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SCIENTIFIC DATA REVIEWS
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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: GLUFOSINATE-AMMONIUM METABOLITES (Hoe 099730 and Hoe 061517) -- Toxicology Data (Subchronic Feeding Studies) Submitted Under MRID Nos. 440762-01, -02, -03, -06, -07 and -08 [§82-1(a) and §82-1(b)]

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Registrant: AgrEvo USA Co.

Chemicals: Glufosinate-ammonium metabolite Hoe 099730 (MRID Nos.440762-01 thru -03
Glufosinate-ammonium metabolite Hoe 061517 (MRID Nos.440762-06 thru -08

Case No.:	039815	Action:	176 RESB NW PRO-OC-NW N-F/F
Submission No.:	S509558	PC Code.:	128850
DP Barcode:	D229929		

Action Requested: Review and evaluate the following six subchronic feeding studies:

CITATIONS for HOE 099730

Tennekes, H., Probst, D., Luetkemeier, H., et al. (1994); Hoe 099730 - Substance Technical (Code: Hoe 099730 00 ZC75 0001) Sub-Chronic Oral Toxicity - 13 Week Feeding Study in Rats - Plus Amendment. RCC, Research & Consulting Co., Ltd., Itingen, SW; RCC Umweltchemie, Ltd, Itingen, SW; BRL Biological Research Laboratories, Ltd., Füllinsdorf, SW; EPS (UK) Ltd., Hereford, England; RCC (UK) Ltd., Hereford, England. Study No. 291093; Report Nos. A48187 & A53387; Study Completion Date: June 28, 1991; Supplement - December 1, 1994; MRID No.:44076201. Unpublished.

Tennekes, H., Schmid, H., Probst, D., Luetkemeier, H., et al. (1994); Hoe 099730 - Substance Technical (Code: Hoe 099730 00 ZC75 0001) Sub-Chronic Oral Toxicity - 13 Week Feeding Study in Mice - Plus Supplement. RCC, Research & Consulting Co., Ltd., Itingen, SW; RCC Umweltchemie, Ag, Itingen, SW; BRL Biological Research Laboratories, Ltd., Füllinsdorf, SW; EPS (UK) Ltd., Hereford, England; RCC (UK) Ltd., Hereford, England. Study No. 291025; Report Nos. A48186 & A53386; Study Completion Date: June 10, 1991; Supplement - December 1, 1994; MRID No.:44076202. Unpublished.

Corney, S.J., Braunhofer, H., Luetkemeier, H., et. al. (1994); Hoe 099730 - Substance Technical (Code: Hoe 099730 00 ZC75 0001) 13-Week Oral Toxicity (Feeding) Study in the Dog - Plus Supplement. RCC, Research & Consulting Co., Ltd., Itingen, SW; RCC Umweltchemie, Ag., Itingen, SW; BRL Biological Research Laboratories, Ltd., Füllinsdorf, SW; RCC (UK) Ltd., Hereford, England. Study No. 291104; Report Nos. A49064 & A53388; Study Completion Date: October 27,1992; Supplement - December 1, 1994; MRID No.:44076203. Unpublished.

CITATIONS for HOE 061517

Ebert, E. and Mayer, D. (1988); Hoe 061517 - Substance Technical (Code: Hoe 061517 0Q ZC99 0003) Subchronic Oral Toxicity (13-Week Feeding Study) in the Wistar Rat. Pharma Research Toxicology and Pathology Hoechst Aktiengesellschaft, Frankfurt am Main, Germany. Study No. 831 & 87.0721: Report No. A40450; Study Completion Date: March 30, 1988; MRID No.:44076206. Unpublished.

Ebert, E. and Leist, K.H. (1989); Hoe 061517 - Substance Technical (Code: Hoe 061517 0Q ZC99 0005) Subchronic Oral Toxicity (13 Week Feeding Study) in the NMRI Mouse. Pharma Research Toxicology and Pathology Hoechst Aktiengesellschaft, Frankfurt am Main, Germany. Study No. 88.0693: Report No. A41762; Study Completion Date: June 16, 1989; MRID No.:44076207. Unpublished.

Brunk, R. (1988); Hoe 061517 - Substance Technical (Code: Hoe 061517 0Q ZC99 0003) Testing for Toxicity by Repeated Oral Administration to Beagle Dogs (3-Month Feeding Study). Pharma Research Toxicology and Pathology Hoechst Aktiengesellschaft, Frankfurt am Main, Germany. Study No. 87.0722: Report No. A39880; Study Completion Date: June 21, 1988; MRID No.:44076208. Unpublished.

CONCLUSIONS: The Executive Summaries for the above studies are provided below; the Data Evaluation Records are attached.

HOE 099730

EXECUTIVE SUMMARY: In a subchronic toxicity study (MRID No.44076201), Hoe 099730 (44.4% solution in water, adjusted to 100% purity) was administered in the diet at dose levels of 400, 2000 or 10,000 ppm (equivalent daily intakes were 0, 29, 147 or 738 mg/kg/day, respectively in males and 0, 32, 162 or 800 mg/kg/day, respectively in females) to Wistar rats. Groups consisted of 20 rats/sex/dose in the vehicle control and mid- and high-treatment groups and 10 rats/ sex in the low-treatment group. Ten rats/sex/dose from the vehicle control and mid- and high-treatment groups were retained following treatment for a 4-week recovery period. In addition to standard testing, samples of livers, kidneys and brains of all animals were analyzed for glutamine synthetase activity.

Treatment with Hoe 99730 had no adverse effects on survival, body weight, food consumption, hematology, clinical chemistry, or urinalysis or cause any gross or microscopic lesions.

The LOEL for glutamine synthetase inhibition is 400 ppm, based on the significant ≈27 or 16% reduction in the activity of this enzyme in male and female livers, respectively, and the ≈20 % reduction in the activity of this enzyme in male kidneys. A NOEL was not established.

This subchronic toxicity study is classified **Acceptable (Nonguideline)** as it is not a required study. It is acceptable for the purposes for which it was intended as a special study.

EXECUTIVE SUMMARY: In a subchronic toxicity study (MRID No.44076202), Hoe 099730 (44.4% solution in water, adjusted to 100% purity) was administered in the diet at dose levels of 500, 2000 or 8000 ppm (equivalent daily intakes were 0, 83, 324 or 1296 mg/kg/day, respectively in males and 0, 110, 436 or 1743 mg/kg/day, respectively in females) to NMRI mice. Groups consisted of 20 mice/sex/dose. In addition to standard testing, samples of livers, kidneys and brains of all animals were also analyzed for glutamine synthetase activity.

Treatment with Hoe 99730 had no adverse effects on survival, body weight, food consumption, hematology, or clinical chemistry or cause any gross or microscopic lesions.

The NOEL is >8000 ppm, based on the absence of a significant toxicological response at the highest dose tested.

The LOEL for glutamine synthetase inhibition is 500 ppm, based on the significant ($p<0.01$) ≥25% reduction in the activity of this enzyme in male an d female kidneys. A NOEL was not established.

This subchronic toxicity study is classified **Acceptable (Nonguideline)** as it is not a required study. It is acceptable for the purposes for which it was intended as a special study.

EXECUTIVE SUMMARY: In a subchronic toxicity study (MRID No.44076203), Hoe 099730 (44.4% solution in water, adjusted to 100% purity) was administered in the diet at dose levels of 500, 2000 or 8000 ppm (equivalent daily intakes were 0, 19, 72 or 289 mg/kg/day, respectively in males and 0, 21, 79 or 300 mg/kg/day, respectively in females) to beagle dogs. Groups consisted of six dogs/sex/dose in the vehicle control and mid- and high-treatment groups and four dogs/sex in the low-treatment group. Two dogs/sex/dose from the vehicle control and mid- and high-treatment groups were retained following treatment for a 4-week recovery period. In addition to standard testing, samples of livers, kidneys and brains of all animals were also analyzed for glutamine synthetase activity.

Treatment with Hoe 99730 had no adverse effects on survival, body weight, hematology, or clinical chemistry or cause any gross or microscopic lesions. **The NOEL is >8000 ppm, based on the absence of a significant toxicological response at the highest dose tested.**

The LOEL for glutamine synthetase inhibition is 500 ppm, based on the significant ($p<0.01$) ≈31% reduction in the activity of this enzyme in male livers. A NOEL was not established.

This subchronic toxicity study is classified **Acceptable (Nonguideline)** as it is not a required study. It is acceptable for the purposes for which it was intended as a special study.

HOE 061517

EXECUTIVE SUMMARY: In a subchronic toxicity study (MRID No.44076206), Hoe 061517 (99.6%) was administered in the diet for 13 weeks to male and female Wistar rats at dose levels of 0, 400, 1600 or 6400 ppm (0, 30, 102 or 420 mg/kg/day, respectively in males and 0, 32, 113, or 439 mg/kg, respectively in females). Groups consisted of 20 rats/sex/dose in the vehicle control and mid- and high-treatment groups and 10 rats/sex in the low-treatment group. Ten rats/sex/dose from the vehicle control and mid- and high-treatment groups were retained following treatment for a 4-week recovery period. In addition to standard testing, neurological examinations were conducted; however, glutamine synthetase activity was not measured.

Treatment with Hoe 061517 had no adverse effects on survival, body weight, hematology, clinical chemistry, urinalysis, or cause any neurological changes. However, marginal increases in the absolute and relative high-dose male liver weights (main and recovery groups), which appeared to correlate with the increased incidence of small Kupffer cell proliferates (≈60% of the animals--both main and recovery high-dose groups versus 20% of controls) and increased reticulocyte counts (25% or 14%), were seen at 6400 ppm. Whether these findings are indicative of a toxicologically significant adverse effect is not clear. **Based on the above findings and in disagreement with the study authors, the LOEL was set at 6400 ppm. The NOEL was established at 1600 ppm.**

This subchronic toxicity study is classified **acceptable (Nonguideline)** as it is not a required study. It is, however, acceptable for the purposes for which it was intended as a special study.

EXECUTIVE SUMMARY: In a subchronic toxicity study (MRID No.44076207), Hoe 061517 (99.8%) was administered in the diet for 13 weeks to male and female NMRI mice at dose levels of 0, 320, 1600, 3200 or 8000 ppm (0, 46, 209, 496 or 1121 mg/kg/day in the males and 0, 47, 220, 561 or 1340 mg/kg/day in the females, respectively. All groups consisted of 10 mice/sex/dose. Glutamine synthetase activity was not measured.

Treatment with Hoe 061517 had no adverse effects on survival, body weight, hematology or urinalysis. However, a marginal but significant increase in the relative high-dose female kidney weight and dose-related significant decreases in serum uric acid levels were observed in the mid- and high-dose males (16 and 40% of control, respectively). In the females, nondose-related ≈33 or 25% decreases in uric acid levels were seen at 3200 or 8000 ppm, respectively. There was, however, no evidence of gross or microscopic lesions associated with exposure to Hoe 061517. Whether the marginal kidney weight changes or decreased uric acid levels are indicative of a toxicologically significant adverse effect is not clear. **In disagreement with the study authors, however, the LOEL was set at 8000 ppm based on the decreased serum uric acid levels. The NOEL was established at 3200 ppm.**

This subchronic toxicity study is classified **acceptable (Nonguideline)** as it is not a required study. It is, however, acceptable for the purposes for which it was intended as a special study.

EXECUTIVE SUMMARY: In a subchronic toxicity study (MRID No.44076208), Hoe 061517 (99.6%) was administered in the diet for ~15 weeks to male and female beagle dogs at dose levels of 100, 400 or 1600 mg/kg (equivalent daily intakes could not be determined because of the inadequacies in the analytical data). Groups consisted of six dogs/sex/dose in the vehicle control and mid- and high-treatment groups and four dogs/sex in the low-treatment group. Two dogs/sex/dose from the vehicle control and mid- and high-treatment groups were retained following treatment for a 4-week recovery period. In addition to standard testing, samples of livers, kidneys and brains of all animals were analyzed for glutamine synthetase activity. Hearing tests, neurological examinations hepatic function tests (i.e., bromsulphthalein retention) and renal function test (i.e., phenolsulfonphthalein elimination) were also conducted.

Treatment with Hoe 061517 had no adverse effects on survival, body weight, hematology, clinical chemistry, urinalysis, liver or renal function, glutamine synthetase activity, or cause hearing disfunction or neurological changes. Similarly, there was no evidence of gross or microscopic lesions associated with exposure to Hoe 061517. **However, neither a LOEL nor a NOEL could be established because of numerous study deficiencies (see Section III, Discussion for details).**

This subchronic toxicity study is classified **Unacceptable (Nonguideline)** as it is not a required study. It is, however, unacceptable for the purposes for which it was intended as a special study.

ATTACHMENTS: DERs

ATTACHMENT

DERs FOR GLUFOSINATE-AMMONIUM METABOLITES
(HOE 0099730 AND HOE 061517)

MRID NOs: 440762-01 thru -03;
440762-06 thru -08

[GLUFOSINATE-AMMONIUM METABOLITE] Subchronic Oral Study 82-1(a)

EPA Reviewer: Nancy McCarroll
Review Section III,
Toxicology Branch II (7509C)
EPA Secondary Reviewer:
Whang Phang, Ph.D.
Review Section III,
Toxicology Branch II (7509C)

Signature: Nancy E. McCarroll
Date: 1/2/97

Signature: Whang Phang
Date: 1/2/97

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity [Feeding]-[Rats]; OPPTS 870.3100 (rodent) [§82-1 (a)]

DP BARCODE: D229929 SUBMISSION CODE: S509558
P.C. CODE: 128850 TOX. CHEM. NO.: 580 I (Metabolite)
MRID No.: 44076201

TEST MATERIAL (PURITY): Hoe 099730 (44.4% solution in water) [**Glufosinate-ammonium metabolite**]

SYNONYMS: Code: Hoe 099730 00 ZC75 0001

CITATION: Tennekes, H., Probst, D., Luetkemeier, H., et al. (1994); Hoe 099730 - Substance Technical (Code: Hoe 099730 00 ZC75 0001) Sub-Chronic Oral Toxicity - 13 Week Feeding Study in Rats - Plus Amendment. RCC, Research & Consulting Co., Ltd., Itingen, SW; RCC Umweltchemie, Ltd, Itingen, SW; BRL Biological Research Laboratories, Ltd., Füllinsdorf, SW; EPS (UK) Ltd., Hereford, England; RCC (UK) Ltd., Hereford, England. Study No. 291093; Report Nos. A48187 & A53387; Study Completion Date: June 28, 1991; Supplement - December 1, 1994; MRID No.:44076201. Unpublished.

SPONSOR: AgrEvo USA Co., Wilmington, DE

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID No.44076201), Hoe 099730 (44.4% solution in water, adjusted to 100% purity) was administered in the diet at dose levels of 400, 2000 or 10,000 ppm (equivalent daily intakes were 0, 29, 147 or 738 mg/kg/day, respectively in males and 0, 32, 162 or 800 mg/kg/day, respectively in females) to Wistar rats. Groups consisted of 20 rats/sex/dose in the vehicle control and mid- and high-treatment groups and 10 rats/ sex in the low-treatment group. Ten rats/sex/dose from the vehicle control and mid- and high-treatment groups were retained following treatment for a 4-week recovery period. In addition to standard testing, samples of livers, kidneys and brains of all animals were analyzed for glutamine synthetase activity.

Treatment with Hoe 99730 had no adverse effects on survival, body

weight, food consumption, hematology, clinical chemistry, or urinalysis or cause any gross or microscopic lesions.

The LOEL for glutamine synthetase inhibition is 400 ppm, based on the significant ≈27 or 16% reduction in the activity of this enzyme in male and female livers, respectively, and the ≈20 % reduction in the activity of this enzyme in male kidneys. A NOEL was not established.

This subchronic toxicity study is classified **Acceptable (Nonguideline)** as it is not a required study. It is acceptable for the purposes for which it was intended as a special study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Hoe 099730 technical
   Description: Dark brown liquid (supplied as a 44.4% solution in water (w/w)
   Lot/Batch #: Batch Nos. 1/90 + 2/90
   Purity of the technical ingredient: 74.7% (HPLC analysis)
   Impurities: 0.5% glufosinate (Hoe 035956)
   Stability of compound: Reported to be stable for 12 months at 25°C.
   CAS #: Not provided.
   Structure: Not provided.
   Other provided information: Dietary concentrations were adjusted to 100% test material.

2. Vehicle and/or positive control: Basal Diet (standard Kliba 343 rat maintenance diet)

3. Test animals: Species: Wistar rats
   Age at study initiation: ≈5 weeks
   Weight at study initiation: 80-84 g (♂); 68-71 g (♀)
   Source: BRL Biological Research Labs, Inc., Fuellinsdorf, SW
   Housing: 5/cage
   Diet: Standard Kliba 343 rat maintenance diet was available ad libitum
   Water: Tap water was available ad libitum

   Environmental conditions:
   Temperature: 22±3°C
   Humidity: 40-70%
   Air changes: 10-15 air changes/hour
   Photoperiod: 12 hour light/dark cycle
   Acclimation period: 7 days

B. STUDY DESIGN:

1. In life dates - start: 2/27/91 end: 6/27-28/91

2. Animal assignment

   Animals were assigned randomly to the test groups shown in Table 1.

TABLE 1: STUDY DESIGN

Test Group	Conc. in Diet (ppm)	Dose to Males (mg/kg/day)	Dose to Females (mg/kg/day)	No.+ of ♂	No.+ of ♀
Control	0	0	0	20	20
Low	400	29.1	31.7	10	10
Mid	2000	146.8	162.0	20	20
High	10,000	737.8	799.9	20	20

+ At the end of treatment, ten males and ten females in the vehicle and mid-and high-dose groups were retained for a 4-week recovery period.

3. Diet preparation and analysis:

Test diets were prepared every 2 weeks by mixing appropriate amounts of test substance with microgranulated basal feed; water was added to aid pelleting. Prepared diets were dried for ≈48 hours with warm air and stored at room temperature. Stability in the diet was determined prior to the start of the study. During the study, homogeneity and actual concentration in the three experimental diets were determined at pretest and in weeks 4 and 12.

Results - Homogeneity Analysis: Within -7% to +5% of mean concentrations.
Stability Analysis: Stable in feed for at least 21 days at room temperature (85.1-98.7% of target concentrations recovered).
Concentration Analysis: 93.1-98.9% of nominal

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. Statistics: Body weight, clinical laboratory data and organ weights were analyzed using a one-way analysis of variance, Dunnett's test and/or Steel's test. Ophthalmoscopy data were evaluated by Fisher's exact test.

C. METHODS:

1. Observations:

Animals were inspected twice daily for mortality and at least once daily for clinical signs. Detailed clinical examinations were conducted weekly.

2. Body weight:

Animals were weighed at pretest and weekly, thereafter, until sacrifice.

3. Food consumption and compound intake:

Food consumption per cage was recorded weekly and mean daily diet consumption was calculated as g food/kg body weight/day. Food efficiency was not determined. Compound intake (mg/kg/day) values were calculated per cage as time-weighted averages from the consumption and body weight data.

4. Ophthalmoscopic examination

Eyes were examined at pretest and at weeks 11 and 16 (recovery animals).

5. Blood was collected for hematology and clinical analysis from all survivors in all groups. Animals were fasted ≈18 hours prior to blood collection at weeks 13 and following the recovery period (week 17). All blood samples were collected between the hours of 6:20 and 7:30 AM. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*		
x	(Thromboplastin time)		
x	(Activated Partial Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical Chemistry:

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
X	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)	X	Triglycerides
	Cholinesterase (ChE)	X	Serum protein electrophores
X	Creatine phosphokinase		
X	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (SGPT)*		
X	Serum aspartate amino-transferase (SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis*

Urine was collected from fasted animals at week 13 and following the recovery period (week 17). The CHECKED (X) parameters were examined.

X	Color		
X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen

* Not required for subchronic studies

7. Residue Analysis:

At end of treatment or the recovery period, blood samples were taken from ten animals per sex per group, centrifuged and separated into plasma and red blood cell fractions. Animals were placed in metabolism cages and urine and feces samples were also collected. All blood, urine and feces samples were held frozen for possible residue analysis.

8. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)[T]
X	Stomach*	X	Lymph nodes*		
X	Duodenum*	XX	Spleen*	XX	Pituitary*
X	Jejunum*	X	Thymus*	X	Eyes (optic n.)[T]
X	Ileum*				
X	Cecum*		UROGENITAL		
X	Colon*	XX	Kidneys*+		GLANDULAR
X	Rectum*	X	Urinary bladder*	XX	Adrenal gland*
XX	Liver*+	XX	Testes*+	X	Lacrimal gland[T]
	Gall bladder*	X	Epididymides	X	Mammary gland[T]
X	Pancreas*	X	Prostate	X	Parathyroids*++
		X	Seminal vesicle	XX	Thyroids*++
	RESPIRATORY	XX	Ovaries		
X	Trachea*	X	Uterus*		OTHER
XX	Lung*	X	Cervix	X	Bone
	Nose	X	Vagina	X	Skeletal muscle
	Pharynx			X	Skin
X	Larynx			X	All gross lesions and masses*

* Required for subchronic studies based on Subdivision F Guidelines
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

9. Additional Testing:

Glutamine Synthetase Activity: Samples of the liver, pooled sections from the left and right kidneys including the cortex and medulla, and the brain from all animals were analyzed for glutamine synthetase (GS). Samples were stored frozen until analysis. The analytical method was colorimetric "based on the gamma-glutamyltransferase reaction catalysed by glutamine synthetase with the formation of gamma-glutamyl hydroxamate". Presented values were expressed as μmol γ-glutamyl-hydroxamate/mL of the reaction mixture.

II. RESULTS

A. Observations :

1. Toxicity - No compound-related clinical signs were observed.

2. Mortality - All animals survived until the scheduled sacrifices.

B. Body weight and weight gain: Treatment with Hoe 099730 had no adverse effect on body weight or body weight gain.

C. Food consumption and compound intake:

Food consumption - Food consumption in the males and females of all dose groups was unaffected by treatment.

Test Article Intake - (time-weighted average): The mean intake values of Hoe 099730 calculated using nominal dietary levels for male rats receiving test diets containing 0, 400, 2000 or 10,000 ppm were 0, 29, 147 or 738 mg/kg/day, respectively. The mean intake values for females receiving these same dietary concentrations were 0, 32, 162 or 800 mg/kg/day, respectively.

Food efficiency - Food efficiency values were not determined.

D. Ophthalmoscopic examination - No treatment-related effects on the appearance of the eyes were observed at any examination period.

E. Blood work:

Hematology - No treatment-related effects on hematology parameters were observed. Marginal but significant changes in the erythrocyte count, hemoglobin concentration and mean corpuscular volume for high-dose females were within the limits of the provided historical control ranges.

Clinical Chemistry - There was no indication of a compound-related effect on clinical chemistry parameters. Although significantly different from control results were recorded for several parameters in both the male and the female rats, the changes were generally very slight, not dose-related and fell within the provided historical control ranges of the performing laboratory.

F. Urinalysis - No treatment-related effects on urinalysis parameters were observed.

G. Additional Studies:

Glutamine Synthetase (GS) Activity:

Treated males showed generally dose-related and significant decreases in liver and kidney GS activity at all levels and in the brain at 2000 and 10,000 ppm (Table 2). In the females, significant and dose-related responses were obtained in the liver at all concentrations and in the brain at 10,000 ppm. GS activity in the female kidneys was unaffected by treatment with H 099730. Data from the recovery groups are also presented in Table 2. As shown, effects on GS activity in the recovery group animals were less pronounced than at the end of the 13-week treatment with Hoe 99730. The study authors stated that since effects on GS activity were reversible following withdrawal of treatment, the results were the consequence of contamination of the test substance with glufosinate, a proven inhibitor of this enzyme in plants and animals.

H. Sacrifice and Pathology:

Organ weight - Representative data presented in Table 3 show that absolute kidney weights for the males of all treatment groups were increased; the response for the high-and low-dose groups (≈15% higher than control) was significant ($p<0.05$) while the 4% increase at 2000 ppm was not significant. Differences in kidney-to-body weight and kidney-to-brain weight were also significant at 10,000 ppm. However, no significant effects on the kidney weights of the recovery group males or the females of any treatment group (primary or recovery groups) were recorded. Results for all other organ weights were comparable to controls.

Gross pathology - No treatment-related necropsy findings were observed.

Microscopic pathology - No treatment-related microscopic findings were observed.

III. **DISCUSSION:**

Review of the final report and supporting data indicated that the conduct and design of the study were adequate and the reporting of the results was accurate. Although increased absolute and relative (organ-to-body and organ-to-brain weights) kidney weights were observed in the high-dose males, these findings were not seen in the males of the recovery groups or the females of any treatment group. Our reviewers noted that transient male kidney weight increases tended to parallel the reversible inhibitory effects on GS activity. However, increased kidney weights were not correlated with gross or

microscopic pathological changes in the kidneys. The data, therefore, support the study authors' assessment that Hoe 099730 did not produce toxicologically significant adverse effects. The study investigators also demonstrated that the inhibition of GS activity seen in the livers of both sexes at all treatment levels, in male kidneys at all treatment doses and in the brains of both sexes at the highest tested concentration (10,000 ppm) was reversible. We further agree with the study authors' statement that "The inhibition of glutamine synthetase activity in animals is an indication of the presence of glufosinate and is considered to be without any toxicological significance."

Our reviewers noted that the Hoe 99730 metabolite at 400 ppm (the equivalent amount of the parent at this concentration would be ≈20 ppm, based on the reported 0.5% glufosinate impurity) caused an ≈27 or 16% inhibition of GS in the livers of the males or females, respectively. In a previously submitted subchronic rat feeding study with the parent compound, Hoe 39866 (see MRID No. 00142444), males receiving a dietary level of 8 ppm Hoe 39866 had liver GS activity that was decreased by 17% relative to control. No inhibition was seen in the females of this dose group; at 64 ppm, however, GS activity in the liver was reduced by 13%. Given the probable differences in analytical methods, the similarity of the response in both studies would appear to support the investigators' further statement that inhibition of GS was probably attributable to the contamination of Hoe 99730 with 0.5% glufosinate.

A NOEL for GS inhibition was, however, not established.

B. Study deficiencies: The following study deficiencies that probably did not alter the outcome of the study were noted:

Although a NOEL was not established, the study is considered **Acceptable** because Hoe 099730 accounts for <10% of the active ingredient.

Our reviewers would have preferred a higher purity of the technical ingredient (74.7%) in the original sample used to prepare the 44.4% solution of Hoe 099730. However, we assume that since Hoe 09973 is a metabolite, technical difficulties may have precluded the preparation of a higher purity.

Table 2. Glutamine Synthetase Activity (μmol γ-glutamyl-hydroxamate/mL reaction mixture) in Wistar Rats Ingesting Hoe 099730 in the Diet for 13 weeks[a,b]

	Dietary Level (ppm)			
Tissue	0	400	2000	10,000
		MALES		
Liver				
Week 13	3.76	2.76**(27%)[c]	2.18**(42%)[c]	1.77**(53%)[c]
Week 17 (Recovery)	3.17	--	3.49	3.42
Kidney				
Week 13	2.10	1.68**(20%)	1.49**(29%)	1.64**(22%)
Week 17 (Recovery)	2.13	--	2.09 (2%)	1.88 (12%)
Brain				
Week 13	3.19	3.26	3.02* (5%)	2.82**(12%)
Week 17 (Recovery)	3.14	--	2.98 (5%)	2.89**(8%)
		FEMALES		
Liver				
Week 13	3.71	3.12* (16%)	2.57**(31%)	2.35**(37%)
Week 17 (Recovery)	3.73	--	3.53 (5%)	3.32* (11%)
Kidney				
Week 13	1.17	1.12	1.20	1.52**†
Week 17 (Recovery)	1.27	--	1.28	1.32
Brain				
Week 13	3.12	3.06 (2%)	3.02 (3%)	2.77**(11%)
Week 17 (Recovery)	3.09	--	3.11	2.92* (6%)

[a] Data extracted from Study # 291093, Clinical Biochemistry Summaries; pp. 97 and 99.
[b] N = 10 for all groups
[c] Values in () represent percent inhibition of glutamine synthetase compared to the corresponding control.

* Significantly different from control ($p<0.05$) by Dunnett's test.
** Significantly different from control ($p<0.01$) by Dunnett's test.

Table 3. Kidney Weights (Absolute and Relative) of Wistar Rats Ingesting Hoe 099730 in the Diet for 13 weeks[a,b]

	Dietary Level (ppm)			
Tissue	0	400	2000	10,000
	MALES (PRIMARY GROUPS)			
Body weight (g)	392.5	423.2	377.4	393.5
Kidney				
Absolute (g)	2.17	2.49*	2.25	2.49*
Relative (to Body Weight %)	0.55	0.59	0.60*	0.63**
Relative (to Brain Weight %)	103.33	117.94*	108.52	119.19*
	MALES (RECOVERY GROUPS)			
Body weight (g)	417.2	--	400.0	410.0
Kidney				
Absolute	2.32	--	2.29	2.45
Relative (to Body Weight %)	0.56	--	0.57	0.60
Relative (to Brain Weight %)	107.84	--	109.01	115.18
	FEMALES (PRIMARY GROUPS)			
Body weight (g)	222.0	213.7	222.2	220.6
Kidney				
Absolute	1.43	1.37	1.44	1.48
Relative (to Body Weight %)	0.64	0.64	0.65	0.67
Relative (to Brain Weight %)	73.40	71.79	74.38	76.65
	FEMALES (RECOVERY GROUPS)			
Body weight (g)	239.6	--	222.0	238.0
Kidney				
Absolute	1.44	--	1.39	1.52
Relative (to Body Weight %)	0.60	--	0.62	0.64
Relative (to Brain Weight %)	74.76	--	70.38	76.55

[a] Data extracted from Study # 291093, Organ Weight Summaries; pp. 102-113.
[b] N = 10 for all groups

* Significantly different from control ($p<0.05$) by Dunnett's test.
** Significantly different from control ($p<0.01$) by Dunnett's test.

[GLUFOSINATE-AMMONIUM METABOLITE] Subchronic Oral Study 82-1(a)

EPA Reviewer: Nancy McCarroll
Review Section III,
Toxicology Branch II (7509C)
EPA Secondary Reviewer:
Whang Phang, Ph.D.
Review Section III,
Toxicology Branch II (7509C)

Signature: Nancy E. McCarroll
Date: 1/15/97

Signature: Whang Phang
Date: 1/15/97

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity [Feeding]-[Mice]; OPPTS 870.3100 (rodent) [§82-1 (a)]

DP BARCODE: D229929 SUBMISSION CODE: S509558
P.C. CODE: 128850 TOX. CHEM. NO.: 580 I (metabolite)
MRID No.: 44076202

TEST MATERIAL (PURITY): Hoe 099730; N-acetyl-L-glufosinate ammonium (44.4% solution in water)

SYNONYMS: Code: Hoe 099730 00 ZC75 0001; **[Glufosinate-ammonium metabolite]**

CITATION: Tennekes, H., Schmid, H., Probst, D., Luetkemeier, H., et al. (1994); Hoe 099730 - Substance Technical (Code: Hoe 099730 00 ZC75 0001) Sub-Chronic Oral Toxicity - 13 Week Feeding Study in Mice - Plus Supplement. RCC, Research & Consulting Co., Ltd., Itingen, SW; RCC Umweltchemie, Ag, Itingen, SW; BRL Biological Research Laboratories, Ltd., Füllinsdorf, SW; EPS (UK) Ltd., Hereford, England; RCC (UK) Ltd., Hereford, England. Study No. 291025; Report Nos. A48186 & A53386; Study Completion Date: June 10, 1991; Supplement - December 1, 1994; MRID No.:44076202. Unpublished.

SPONSOR: AgrEvo USA Co., Wilmington, DE

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID No.44076202), Hoe 099730 (44.4% solution in water, adjusted to 100% purity) was administered in the diet at dose levels of 500, 2000 or 8000 ppm (equivalent daily intakes were 0, 83, 324 or 1296 mg/kg/day, respectively in males and 0, 110, 436 or 1743 mg/kg/day, respectively in females) to NMRI mice. Groups consisted of 20 mice/sex/dose. In addition to standard testing, samples of livers, kidneys and brains of all animals were also analyzed for glutamine synthetase activity.

Treatment with Hoe 99730 had no adverse effects on survival, body weight, food consumption, hematology, or clinical chemistry or cause any gross or microscopic lesions.

The NOEL is >8000 ppm, based on the absence of a significant toxicological response at the highest dose tested.

The LOEL for glutamine synthetase inhibition is 500 ppm, based on the significant ($p<0.01$) ≥25% reduction in the activity of this enzyme in male an d female kidneys. A NOEL was not established.

This subchronic toxicity study is classified **Acceptable (Nonguideline)** as it is not a required study. It is acceptable for the purposes for which it was intended as a special study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Hoe 099730 technical
   Description: Dark brown liquid supplied as a 44.4% solution in water (w/w)).
   Lot/Batch #: Batch Nos. 1/90 + 2/90
   Purity of the technical ingredient: 74.7% (HPLC analysis)
   Impurities: 0.5% glufosinate (Hoe 035956) (parent compound)
   Stability of compound: Reported to be stable for 12 months at 25°C.
   CAS #: Not provided.
   Structure: Not provided.
   Other provided information: Dietary concentrations were adjusted to 100% test material.

2. Vehicle and/or positive control: Basal Diet (standard Kliba 343 mouse maintenance diet)

3. Test animals: Species: NMRI mice
   Age at study initiation: ≈5 weeks
   Weight at study initiation: 17.1-18.4 g (♂); 15.2-15.6 g (♀)
   Source: BRL Biological Research Labs, Inc., Füllinsdorf, SW
   Housing: Individual
   Diet: Standard Kliba 343 mouse maintenance diet was available ad libitum
   Water: Tap water was available ad libitum

   Environmental conditions:
   Temperature: 22±3°C
   Humidity: 40-70%
   Air changes: 10-15 air changes/hour
   Photoperiod: 12 hour light/dark cycle
   Acclimation period: 7 days

B. STUDY DESIGN:

1. In life dates - start: 3/5/91 end: 5/6, 7, 10/91

2. Animal assignment

   Animals were assigned randomly to the test groups shown in Table 1.

TABLE 1: STUDY DESIGN

Test Group	Conc. in Diet (ppm)	Dose to Males (mg/kg/day)	Dose to Females (mg/kg/day)	No. of ♂	No. of ♀
Control	0	0	0	20	20
Low	500	82.9	109.7	20	20
Mid	2000	323.5	435.7	20	20
High	8000	1296.1	1743.3	20	20

3. Diet preparation and analysis:

Test diets were prepared every 2 weeks by mixing appropriate amounts of test substance with microgranulated basal feed; water was added to aid pelleting. Prepared diets were dried for ≈48 hours with warm air and stored at room temperature. Stability in the diet was determined at the start of the study. During the study, homogeneity and actual concentrations in the three experimental diets were determined on the first and last feed batches prepared for the study.

Results - Homogeneity Analysis: Within -7% to +6% of mean concentrations.
Stability Analysis: Stable in feed for at least 21 days at room temperature.
Concentration Analysis: 95.8-99.0% of nominal

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. Statistics: Body weight, food consumption, clinical laboratory data and organ weights were analyzed using a one-way analysis of variance, Dunnett's test and/or Steel's test.

C. METHODS:

1. Observations:

Animals were inspected twice daily for mortality and at least once daily for clinical signs. Detailed clinical examinations were conducted weekly.

2. Body weight:

Animals were weighed at pretest and weekly, thereafter, until sacrifice.

3. Food consumption and compound intake:

Food consumption was recorded weekly and mean daily diet consumption was calculated as g food/kg body weight/day. Food efficiency was not determined. Compound intake (mg/kg/day) values were calculated as time-weighted averages from the consumption and body weight data.

4. Ophthalmoscopic examination

Eyes were not examined.

5. Blood was collected for hematology and clinical analysis from 10 animals/sex/group at weeks 13. Animals were fasted for ≈18 hours before blood collection and samples were taken between 6:45 and 8:00 AM. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc. (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements* (Thromboplastin time) (Activated Partial Thromboplastin time) (Clotting time) (Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

Note: Blood clotting measurements were not performed. Whang is this okay?

b. Clinical Chemistry:

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
X	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)	X	Triglycerides
	Cholinesterase (ChE)	X	Serum protein electrophores
X	Creatine phosphokinase		
X	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (SGPT)*		
X	Serum aspartate amino-transferase (SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines
Note: Chlorine levels were not determined.

6. Urinalysis*: Urinalysis was not performed.

7. Sacrifice and Pathology: All animals that died and those sacrificed on schedule were subjected to gross pathological examination, and the CHECKED (X) tissues listed below were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*Periph.
X	Salivary glands*	XX	Heart*	X	nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)[T]
X	Stomach*	X	Lymph nodes*		
X	Duodenum*	XX	Spleen*	XX	Pituitary*
X	Jejunum*	X	Thymus*	X	Eyes (optic n.)[T]
X	Ileum*				
X	Cecum*		UROGENITAL		
X	Colon*	XX	Kidneys*+		GLANDULAR
X	Rectum*	X	Urinary bladder*	XX	Adrenal gland*
XX	Liver*+	XX	Testes*+	X	Lacrimal gland[T]
X	Gall bladder*	X	Epididymides	X	Mammary gland[T]
X	Pancreas*	X	Prostate	X	Parathyroids*++
		X	Seminal vesicle	XX	Thyroids*++
	RESPIRATORY	XX	Ovaries		
X	Trachea*	X	Uterus*		
XX	Lung*				OTHER
	Nose			X	Bone
	Pharynx			X	Skeletal muscle
X	Larynx			X	Skin
				X	All gross lesions and masses*

* Required for subchronic studies based on Subdivision F Guidelines
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

9. Additional Testing:

Glutamine Synthetase Activity: Samples of the liver, pooled sections from the left and right kidneys including the cortex and medulla, and brain tissue (one-half) from all animals were analyzed for glutamine synthetase. Samples were stored frozen until analysis. Kidney and brain samples from 2 animals per sex per group were pooled to assure proper enzyme determinations. The analytical method was colorimetric "based on the gamma-glutamyltransferase reaction catalysed by glutamine synthetase with the formation of gamma-glutamyl hydroxamate". Presented values were expressed as μmol γ-glutamyl-hydroxamate/mL of the reaction mixture.

II. RESULTS

A. Observations :

1. Toxicity - No compound-related clinical signs were observed.

2. Mortality - One high-dose male died after blood collection at week 13. This death was not considered to be related to

compound exposure. In addition, one vehicle control female died in the final treatment week from unknown causes.

B. Body weight and weight gain: Treatment with Hoe 099730 had no adverse effect on body weight.

C. Food consumption and compound intake:

1. Food consumption - In general, food consumption in the males and females of all dose groups was unaffected by treatment.

2. Test Article Intake -(time-weighted average): The mean intake values of Hoe 099730 calculated using nominal dietary levels for male rats receiving test diets containing 0, 500, 2000 or 8000 ppm were 0, 83, 324 or 1297 mg/kg/day, respectively. The mean intake values for females receiving these same dietary concentrations were 0, 110, 436 or 1743 mg/kg/day, respectively.

3. Food efficiency - Food efficiency values were not determined.

D. Blood work:

Hematology - No treatment-related effects on hematology parameters were observed. Significant changes in the erythrocyte count, hematocrit and differential leucocyte count for males were sporadic, not dose-related and within the limits of the provided historical control ranges.

Clinical Chemistry - There was no indication of a compound-related effect on clinical chemistry parameters. Marginal but significant changes were not dose-related and largely confined to males and females in the 500-ppm group.

E. Glutamine Synthetase Activity: Treated males showed dose-related and significant inhibition of liver glutamine synthetase activity at 2000 and 8000 ppm and significant and dose dependent decreased kidney and brain glutamine synthetase activity at all levels (Table 2). Findings were generally comparable in the females. The study authors stated that effects on glutamine synthetase activity were the consequence of contamination of the test substance with glufosinate ammonium (parent compound), a proven inhibitor of this enzyme in plants and animals.

H. Sacrifice and Pathology:

Organ weight - Organ weights were unaffected by 13 weeks of treatment with Hoe 099730.

Gross pathology - No treatment-related necropsy findings were seen.

Microscopic pathology - No treatment-related microscopic findings were observed.

III. **DISCUSSION:**

Review of the final report and supporting data indicated that the conduct and design of the study were adequate and the reporting of the results was accurate. Inhibition of glutamine synthetase activity was seen in the kidney of both sexes at all treatment levels and in the liver and brain of both sexes at 2000 and 8000 ppm. The study authors' assessment that the inhibition of glutamine synthetase activity in animals is an indication of the presence of glufosinate ammonium is supported by the findings of other subchronic oral toxicity studies performed with Hoe 099730 in dogs (MRID No. 44076203) and in rats (MRID No. 44076201). In these studies, inhibitory effects on glutamine synthetase were shown to be reversible, without toxicological significance, and probably attributable to the contamination of Hoe 99730 with 0.5% glufosinate. Hence, the data from the this 13-week feeding study in mice suggest that Hoe 099730 did not produce toxicologically significant adverse effects.

The NOEL for this study is, therefore, set at >8000 ppm based on the absence of a significant toxicological response at the highest dose tested.

A NOEL for glutamine synthetase inhibition was not established.

B. Study deficiencies: The following study deficiencies that probably did not alter the outcome of the study were noted:

Although a NOEL was not established, the study is considered scientifically valid and Acceptable because Hoe 099730 accounts for <10% of the active ingredient.

Our reviewers would have preferred a higher purity of the technical ingredient (74.7%) in the original sample used to prepare the 44.4% solution of Hoe 099730. However, we assume that since Hoe 09973 is a metabolite, technical difficulties may have precluded the preparation of a higher purity.

Finally, the omission of several subdivision F recommended parameters (i.e., ophthalmoscopic examinations, blood clotting measurements and chlorine determinations) did not compromise the study findings. Based on the results of other subchronic studies with the compound showing that these parameters were unaffected by treatment, we conclude that the interpretation of the data was not altered by this deficiency.

Table 2. Glutamine Synthetase Activity (μmol γ-glutamyl-hydroxamate/mL reaction mixture) in NMRI Mice Ingesting Hoe 099730 in the Diet for 13 weeks[a]

Tissue	Dietary Level (ppm) 0	500	2000	8000
		MALES		
Liver[b]				
Week 13	3.98	3.74 (6%)	3.34**(16%)[c]	2.94**(26%)[c]
Kidney[d]				
Week 13	1.42	1.01**(29%)	0.83**(42%)	0.71**(50%)
Brain[d]				
Week 13	3.57	3.32**(7%)	3.17**(11%)	2.59**(28%)
		FEMALES		
Liver[b]				
Week 13	4.97	4.91 (1%)	4.60* (7%)	3.98**(20%)
Kidney[d]				
Week 13	1.73	1.29* (25%)	1.20**(31)	1.10**(36%)
Brain[d]				
Week 13	3.38	3.32 (2%)	2.94**(13%)	2.18**(36%)

[a] Data extracted from Study # 291025, Clinical Biochemistry Summaries; pp. 73 and 74, and individual animal data, pp. 149-156.

[b] N = 20 for liver determinations with the exception of high-dose males and vehicle control females. Owing to single unscheduled deaths in these groups, N = 19.

[c] Values in () represent percent inhibition of glutamine synthetase compared to the corresponding control.

[d] Results from pooled kidney and pooled brain samples of 2 animals/sex/group with the exception of high-dose males and vehicle control females. Owing to single unscheduled deaths in these groups, one set of values was derived from single animals.

* Significantly different from control ($p < 0.05$) by Dunnett's test.
** Significantly different from control ($p < 0.01$) by Dunnett's test.

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[GLUFOSINATE-AMMONIUM METABOLITE] Subchronic Oral Study 82-1(b)

EPA Reviewer: Nancy McCarroll
Review Section III,
Toxicology Branch II (7509C)
EPA Secondary Reviewer:
Whang Phang, Ph.D.
Review Section III,
Toxicology Branch II (7509C)

Signature: Nancy E. McCarroll
Date: 1/2/97

Signature: W Phang
Date: 1/2/97

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity [Feeding] - [Beagle Dogs]; OPPTS 870.3151 (nonrodent) [§82-1 (b)]

DP BARCODE: D229929 SUBMISSION CODE: S509558
P.C. CODE: 128850 TOX. CHEM. NO.: 580 I (Metabolite)
MRID No.: 44076203

TEST MATERIAL (PURITY): Hoe 099730 (44.4% solution in water) [Glufosinate-ammonium metabolite]

SYNONYMS: Code: Hoe 099730 00 ZC75 0001

CITATION: Corney, S.J., Braunhofer, H., Luetkemeier, H., et. al. (1994); Hoe 099730 - Substance Technical (Code: Hoe 099730 00 ZC75 0001) 13-Week Oral Toxicity (Feeding) Study in the Dog - Plus Supplement. RCC, Research & Consulting Co., Ltd., Itingen, SW; RCC Umweltchemie, Ag., Itingen, SW; BRL Biological Research Laboratories, Ltd., Füllinsdorf, SW; RCC (UK) Ltd., Hereford, England. Study No. 291104; Report Nos. A49064 & A53388; Study Completion Date: October 27,1992; Supplement - December 1, 1994; MRID No.:44076203. Unpublished.

SPONSOR: AgrEvo USA Co., Wilmington, DE

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID No.44076203), Hoe 099730 (44.4% solution in water, adjusted to 100% purity) was administered in the diet at dose levels of 500, 2000 or 8000 ppm (equivalent daily intakes were 0, 19, 72 or 289 mg/kg/day, respectively in males and 0, 21, 79 or 300 mg/kg/day, respectively in females) to beagle dogs. Groups consisted of six dogs/sex/dose in the vehicle control and mid- and high-treatment groups and four dogs/sex in the low-treatment group. Two dogs/sex/dose from the vehicle control and mid- and high-treatment groups were retained following treatment for a 4-week recovery period. In addition to standard testing, samples of livers, kidneys and brains of all animals were also analyzed for glutamine synthetase activity.

Treatment with Hoe 99730 had no adverse effects on survival, body weight, hematology, or clinical chemistry or cause any gross or microscopic lesions. **The NOEL is >8000 ppm, based on the absence**

of a significant toxicological response at the highest dose tested.

The LOEL for glutamine synthetase inhibition is 500 ppm, based on the significant ($p<0.01$) ≈31% reduction in the activity of this enzyme in male livers. A NOEL was not established.

This subchronic toxicity study is classified **Acceptable (Nonguideline)** as it is not a required study. It is acceptable for the purposes for which it was intended as a special study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Hoe 099730 technical
   Description: Dark brown liquid supplied as a 44.4% solution in water (w/w)).
   Lot/Batch #: Batch Nos. 1/90 and 2/90
   Purity of the technical ingredient: 74.7% (HPLC analysis)
   Impurities: 0.5% glufosinate (Hoe 035956)
   Stability of compound: Reported to be stable for at least 17 months at 25°C.
   CAS #: Not listed.
   Structure: Not provided.
   Other provided information: Dietary concentrations were adjusted to 100% test material.

2. Vehicle and/or positive control: Basal Diet (standard Kliba 335 dog maintenance diet)

3. Test animals: Species: pure bred beagle dogs
   Age at study initiation: ≈6-7½ months
   Weight at study initiation: 9.0-9.2 kg (♂); 7.6-8.1 kg (♀)
   Source: Marshall Farms, North Rose, NY
   Housing: Individual kennels
   Diet: Standard Kliba 335 dog maintenance diet was available daily from 10:00 AM to 1:00 PM
   Water: Tap water was available ad libitum

   Environmental conditions:
   Temperature: 22±3°C
   Humidity: 40-70%
   Air changes: 10-15 air changes/hour
   Photoperiod: 12 hour light/dark cycle
   Acclimation period: 5 weeks

B. STUDY DESIGN:

1. In life dates - start: 2/18/91 end: 6/24/91

2. Animal assignment

Animals were assigned randomly to the test groups in Table 1.

TABLE 1: STUDY DESIGN

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg/day)	Male	Female
Control	0	0	6	6
Low	500	20	4	4
Mid	2000	76	6	6
High	8000	294	6	6

Note: At the end of treatment, two males and two females in the vehicle and mid-and high-dose groups were retained for a 4-week recovery period.

3. Diet preparation and analysis:

Test diets were prepared every 2 weeks by mixing appropriate amounts of test substance with microgranulated basal feed; water was added to aid pelleting. Prepared diets were dried for ≈48 hours with warm air and stored at room temperature. Stability in the diet was determined prior to the start of the study and at initiation. During the study, homogeneity and actual concentrations in the three experimental diets were determined twice (diets prepared for weeks 5-6 and 9-10 were analyzed).

Results - Homogeneity Analysis: Within -5% to +7% of mean concentrations.
Stability Analysis: Stable in feed for at least 21 days at room temperature (93.3-105.2% of target concentrations recovered).
Concentration Analysis: 96.2-100.1% of nominal

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. Statistics: Body weight, clinical laboratory data and organ weights were analyzed using a one-way analysis of variance, Dunnett's test and/or Steel's test.

C. METHODS:

1. Observations:

Animals were inspected twice daily for signs of toxicity and mortality.

2. Body weight:

Animals were weighed at pretest and weekly, thereafter, until sacrifice.

3. Food consumption and compound intake:

Food consumption for each animal was recorded daily and mean daily diet consumption was calculated as g food/kg body weight/day. Food efficiency was not determined. Compound intake (mg/kg/day) values were calculated as time-weighted averages from the consumption and body weight data.

4. Ophthalmoscopic examination

Eyes were examined at pretest, weeks 4 and 13, and following the recovery period.

5. Blood was collected for hematology and clinical analysis from all survivors in all groups. Animals were fasted for ≈ xx hours prior to blood collection at pretest, weeks 4 and 13, and following the recovery period at week 17. All blood samples were collected between the hours of 7:00 and 9:00 AM. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc. (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*		
x	(Thromboplastin time)		
x	(Activated Partial Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical Chemistry:

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
X	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
X	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)	X	Triglycerides
	Cholinesterase (ChE)	X	Serum protein electrophores
X	Creatine phosphokinase		
X	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (SGPT)*		
X	Serum aspartate amino-transferase (SGOT)*		
X	Gamma glutamyl transferase (GGT)		
X	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

Iron, total lipids, phospholipids and ornithine carbamyl-transferase were also measured.

6. Urinalysis*

Urine was collected from fasted animals at pretest, weeks 4 and 13, and following the recovery period (week 17). The CHECKED (X) parameters were examined.

X	Appearance	X	Glucose
	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen

* Not required for subchronic studies
The color and osmolality of the urine samples was also recorded.

7. Residue Analysis:

At end of treatment or the recovery period, blood samples were taken from mid-and high-dose animals, centrifuged and separated into plasma and red blood cell fractions. Animals were placed in metabolism cages and urine and feces samples were collected. All blood, urine and feces samples were held frozen for possible future residue analysis.

8. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*Periph.
X	Salivary glands*	XX	Heart*	X	nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)[T]
X	Stomach*	X	Lymph nodes*		
X	Duodenum*	XX	Spleen*	XX	Pituitary*
X	Jejunum*	X	Thymus*	X	Eyes (optic n.)[T]
X	Ileum*				
X	Cecum*		UROGENITAL		
X	Colon*	XX	Kidneys*+		GLANDULAR
X	Rectum*	X	Urinary bladder*	XX	Adrenal gland*
XX	Liver*[+]	XX	Testes*[+]		Lacrimal gland[T]
X	Gall bladder*	X	Epididymides	X	Mammary gland[T]
X	Pancreas*	XX	Prostate	XX	Parathyroids*[++]
			Seminal vesicle	XX	Thyroids*[++]
	RESPIRATORY	X	Ovaries		
X	Trachea*	X	Uterus*		OTHER
X	Lung*			X	Bone
	Nose			X	Skeletal muscle
	Pharynx			X	Skin
	Larynx			X	All gross lesions and masses*

* Required for subchronic studies based on Subdivision F Guidelines
[+] Organ weight required in subchronic and chronic studies.
[++] Organ weight required for non-rodent studies.
T = required only when toxicity or target organ.

9. Additional Testing:

Glutamine Synthetase Activity: Samples of the left lobe of the liver, pooled sections from the left and right kidneys including the cortex and medulla, and four regions of the brain (cortex, midbrain including thalamus, cerebellum and brain stem) for all animals were analyzed for glutamine synthetase (GS). Samples were stored frozen until analysis. The analytical method was colorimetric "based on the gamma-glutamyltransferase reaction catalysed by glutamine synthetase with the formation of gamma-glutamyl hydroxamate". Presented values were expressed as μmol γ-glutamyl-hydroxamate/mL of the reaction mixture.

Tissue Level Determinations: Liver sections and the remainder of the kidneys and brains of mid- and high-dose animals were held frozen for possible future analysis.

II. **RESULTS**

A. Observations :

1. Toxicity - No compound-related clinical signs were observed.

2. Mortality - All animals survived until the scheduled sacrifices.

B. Body weight and weight gain: Treatment with Hoe 099730 had no adverse effect on body weight or body weight gain.

C. Food consumption and compound intake:

1. Food consumption - Food consumption in the males and females of all dose groups was unaffected by treatment. Although food consumption among females receivng 8000 ppm was slightly lower than the controls (mean ≈9%) throughout the course of the study, the decrease was not statistically significant and mostly occurred during the first 5 weeks of the study.

2. Test Article Intake -(time-weighted average): The mean intake values of Hoe 099730 calculated using nominal dietary levels for male dogs receiving test diets containing 0, 500, 2000 or 8000 ppm were 0, 19, 72 or 289 mg/kg/day, respectively. The mean intake values for females receiving these same dietary concentrations were 0, 21, 80 or 300 mg/kg/day, respectively.

3. Food efficiency - Food efficiency values were not determined.

D. Ophthalmoscopic examination - No treatment-related effects on the appearance of the eyes were observed at any examination period.

E. Blood work:

Hematology - No treatment-related effects on hematology parameters were observed. Moderate anemia was found in one mid-dose female of the recovery group; however, this finding was not considered to be treatment-related.

Clinical Chemistry - There was no indication of a compound-related effect on clinical chemistry parameters. Although significantly different from control results were recorded for several parameters in both the male and the female dogs, the values were either not dose-related or fell within the provided historical control ranges of the performing laboratory.

F. Urinalysis - The only observed effects were a minimal (≈≤2%) reduction in the specific gravity and an ≈40% or greater decrease in osmolality for females in the 8000-ppm group at weeks 4 and 13 and following the recovery period. The values for both parameters were significant for the 4- and 13-week samples.

G. Additional Studies:

Glutamine Synthetase (GS) Activity:

Both treated males and females showed dose-related decreases in liver GS activity (Tables 2a and b). Significant responses were obtained in the liver at all doses in the males and at 2000 and 8000 ppm in the females. GS activity in the kidneys was unaffected by treatment with H 099730. Effects on the brain were as follows:

Cortex: ≥10% inhibition at ≥2000 ppm (♂) or at 8000 ppm (♀); with a significant ($p<0.05$) response at 8000 ppm (♂).

Midbrain: Significantly ($p<0.05$) decreased activity (♂) at 8000 ppm; nonsignificant but dose-related ≥12% decreases at all exposure levels (♀).

Cerebellum: Significant and dose-related ≥16% inhibition at 2000 and 8000 ppm (both sexes).

Brain stem: Significant and dose-related ≥24% inhibition at 2000 and 8000 ppm (both sexes).

Data from the recovery groups are also presented in Tables 2a and b. As shown, non-significant but higher than control (generally by ≥10%) percentages of GS inhibition were seen (liver, cortex, cerebellum and brain stem--both sexes and both treatment levels and midbrain--♀ at 8000 ppm) in the recovery group animals. However, effects on GS activity were generally

less pronounced than at the end of the 13-week treatment with Hoe 99730. The study authors stated that since effects on GS activity were reversible following withdrawal of treatment, the results were the consequence of contamination of the test substance with glufosinate, a proven inhibitor of this enzyme in plants and animals.

Tissue Level Determinations: Not performed.

H. Sacrifice and Pathology:

Organ weight - Absolute prostate gland weights for all treatment groups were lower than control; the response was dose-related (range 31-43% less than control) and significant ($p<0.05$) at 8000 ppm. Difference in prostate-to-body weight were not significant but prostate-to-brain weight reductions for all groups were significant ($p<0.05$). In the recovery group males, no significant prostate gland weight decreases were observed; however, there was an ≈50% reduction in the mean absolute and relative weights (organ-to-body weight and organ-to-brain weight ratios) in the two high-dose males. Results for the two males in the 2000-mg/kg group were comparable to control.

Gross pathology - No treatment-related necropsy findings were observed.

Microscopic pathology - No treatment-related microscopic findings were observed.

III. DISCUSSION:

Review of the final report and supporting data indicated that the conduct and design of the study were adequate and the reporting of the results was accurate. The overall results provided convincing evidence that Hoe 099730 did not produce toxicologically significant adverse effects. Although decreased absolute and relative (organ-to-brain) prostate weights were seen in all treatment groups, these findings were not correlated with pathological changes. Similarly, the data support the study authors' assessment that the inhibition of GS activity seen in both sexes at high concentrations (≥2000 ppm) in the liver and regions of the brain, and also in the livers of males and probably females at 500 ppm was reversible. We further agree with the study authors' statement that "The inhibition of glutamine synthetase activity in animals is an indication of the presence of glufosinate and is considered to be without any toxicological significance." There is also evidence from a previously submitted subchronic rat feeding study with the parent compound, Hoe 39866 (see MRID No. 00142444) which suggests that the parent compound rather than the Hoe 09973 metabolite is probably responsible for the inhibition of GS. In

this study, dietary levels as low as 8 ppm Hoe 39866 caused decreased liver GS activity (17%) in the male rats while inhibition of liver GS was seen in the females at 64 ppm (13%). In the currently reviewed study, 31% (males) or 12% (females) liver GS inhibition was recorded at 500 ppm (equivalent to approximately 25 ppm parent compound, based on the reported 0.5% glufosinate impurity). Given the probable differences in analytical methods between the two studies as well as the differences in animal species, the similarity of the response would appear to support the assumption that inhibition of GS was probably attributable to the contamination of Hoe 99730 with 0.5% glufosinate.

The NOEL for this study is, therefore, set at >8000 ppm based on the absence of a significant toxicological response at the highest dose tested.

A NOEL for GS inhibition was not established.

B. Study deficiencies: The following study deficiencies that probably did not alter the outcome of the study were noted:

Although a NOEL was not established, the study is considered scientifically valid and acceptable because Hoe 099730 accounts for <10% of the active ingredient.

Our reviewers would have preferred a higher purity of the technical ingredient (74.7%) in the original sample used to prepare the 44.4% solution of Hoe 099730. However, we assume that since Hoe 09973 is a metabolite, technical difficulties may have precluded the preparation of a higher purity.

[GLUFOSINATE-AMMONIUM METABOLITE] Subchronic Oral Study 82-1(a)

EPA Reviewer: Nancy McCarroll
Review Section III,
Toxicology Branch II (7509C)
EPA Secondary Reviewer:
Whang Phang, Ph.D.
Review Section III,
Toxicology Branch II (7509C)

Signature: Nancy McCarroll
Date: 1/15/97

Signature: Whang Phang
Date: 1/22/97

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity [Feeding]-[Rats]; OPPTS 870.3100 (rodent) [§82-1 (a)]

DP BARCODE: D232426 (sub-bean to D229929) SUBMISSION CODE: S509558
P.C. CODE: 128850 TOX. CHEM. NO.: 580I (Metabolite)
MRID No.: 44076206

TEST MATERIAL (PURITY): Hoe 061517 (99.6%)

SYNONYMS: Code: Hoe 061517 0Q ZC99 0003; 3-methylphosphinico-propionic acid; metabolite of Hoe 039866 (glufosinate-ammonium)

CITATION: Ebert, E. and Mayer, D. (1988); Hoe 061517 - Substance Technical (Code: Hoe 061517 0Q ZC99 0003) Subchronic Oral Toxicity (13-Week Feeding Study) in the Wistar Rat. Pharma Research Toxicology and Pathology Hoechst Aktiengesellschaft, Frankfurt am Main, Germany. Study No. 831 & 87.0721: Report No. A40450; Study Completion Date: March 30, 1988; MRID No.:44076206. Unpublished.

SPONSOR: AgrEvo USA Co., Wilmington, DE

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID No.44076206), Hoe 061517 (99.6%) was administered in the diet for 13 weeks to male and female Wistar rats at dose levels of 0, 400, 1600 or 6400 ppm (0, 30, 102 or 420 mg/kg/day, respectively in males and 0, 32, 113, or 439 mg/kg, respectively in females). Groups consisted of 20 rats/sex/dose in the vehicle control and mid- and high-treatment groups and 10 rats/sex in the low-treatment group. Ten rats/sex/dose from the vehicle control and mid- and high-treatment groups were retained following treatment for a 4-week recovery period. In addition to standard testing, neurological examinations were conducted; however, glutamine synthetase activity was not measured.

Treatment with Hoe 061517 had no adverse effects on survival, body weight, hematology, clinical chemistry, urinalysis, or cause any neurological changes. However, marginal increases in the absolute

and relative high-dose male liver weights (main and recovery groups), which appeared to correlate with the increased incidence of small Kupffer cell proliferates (≈60% of the animals--both main and recovery high-dose groups versus 20% of controls) and increased reticulocyte counts (25% or 14%), were seen at 6400 ppm. Whether these findings are indicative of a toxicologically significant adverse effect is not clear. **Based on the above findings and in disagreement with the study authors, the LOEL was set at 6400 ppm. The NOEL was established at 1600 ppm.**

This subchronic toxicity study is classified **acceptable (Nonguideline)** as it is not a required study. It is, however, acceptable for the purposes for which it was intended as a special study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Hoe 061517 technical
   Description: Not listed
   Lot/Batch #: Sample No. Roe 15109
   Purity: 99.6%
   Stability of compound: Reported to be stable in the diet for 30 days.
   CAS #: Not provided.
   Structure: Not provided.
   Other provided information: None

2. Vehicle and/or positive control: Basal diet (Altromin 1321 rat diet)

3. Test animals: Species: Wistar rats strain: Hoe:WISKf(SPF71)
   Age at study initiation (range-finding study): ≈5 weeks
   Weight (study initiation): 83.0 ± 0.82 g (♂); 86.0 ±1.41 g (♀)
   Source: Hoechst AG, Pharma Res. Tox., Kastengrund, Ger.
   Housing: Individual
   Diet: Altromin 1321 rat diet was available ad libitum, except during urine sample collection.
   Water: Tap water was available ad libitum, except during urine sample collection.

   Environmental conditions:
   Temperature: 20-24°C
   Humidity: 30-70%
   Air changes: 6 times/hour
   Photoperiod: 12 hours/day
   Acclimation period: One week

B. STUDY DESIGN:

1. In life dates - start: 8/14-19/87 end: 12/10-15/87

2. Animal assignment

   Animals were assigned randomly to the test groups shown in Table 1.

TABLE 1: STUDY DESIGN

Test Group	Conc. in Diet (ppm)	Dose to Males (mg/kg/day)	Dose to Females (mg/kg/day)	No.[a] of ♂	No.[a] of ♀
Control	0	0	0	20	20
Low	400	30[b]	32[b]	10	10
Mid	1600	102	113	20	20
High	6400	420	439	20	20

[a] At the end of treatment, ten males and ten females in the vehicle and mid-and high-dose groups were retained for a 4-week recovery period.

[b] Actual dosages to the animals based on daily intake and analytical determinations (calculated by our reviewers).

3. Diet preparation and analysis:

Premix batches of the test material in basal feed were prepared monthly and stored at room temperature. Final mixtures were prepared monthly by blending the appropriate amount of premix with the rat feed. Each final mixture was analyzed for actual concentration. Stability at days 1 and 30 and homogeneity were measured once on the initial batch of the test diets.

Results -

Homogeneity/Stability Analysis:
Day 1: 72-82%
Day 30: 74-97%

Concentration Analysis:

	Range	Average Values
Low-dose:	82-97% of nominal	88%
Mid-dose:	77-81% of nominal	80%
High-dose:	72-84% of nominal	77%

The analytical data indicated that the mixing procedures were adequate to determine the actual concentration of the test substance. However, the variance between nominal and actual dosage to the animals (77-88%) was unacceptable. Therefore, the average values calculated by our reviewers were used to determine actual dosages to the animals.

4. Statistics: Body weight and water consumption data were evaluated using the parametric method of Sidak, the distribution free method of Nemenyi/Sidak and/or by the parametric method of Dunnett. Organ weight data (absolute and relative) were analyzed using the parametric method of Dunnett, the parametric method of Sidak, and/or the distribution free method of Nemenyi/Dunnett. Hematology data were evaluated using the parametric method of Dunnett, the parametric method of Sidak, the distribution free method of Nemenyi/Dunnett and/or the distribution-free method of Nemenyi/Sidak. Similar statistical procedures were used to analyze the clinical chemistry data and the urinalysis data (volume and pH only). In all cases, significance was established at $p<0.05$.

C. METHODS:

1. Dose selection: Doses selected for the 13-week feeding study were determined based on the results of a preliminary 28-day feeding study in rats (Study Report No. 86.0012) conducted with 0, 50, 500. or 5000 mg/kg of Hoe 061517. The study authors indicated that "no findings of toxicological significance could be established" in the range-finding study. They did, however, note that a "marginal" increase in the liver weight of the high-dose females occurred but the increase was not correlated with any other pathological changes in the liver.

2. Observations:

Animals were inspected for mortality and behavior twice daily. Other signs of toxicity including neurological disturbances, opacity of the refracting media of the eyes, damage to the oral mucosa, and impairment of dental growth were monitored weekly.

3. Body weight:

Animals were weighed once during the acclimation period and weekly, thereafter, throughout the course of study.

4. Food consumption and compound intake:

Food consumption for each animal was determined continuously (weighing once weekly) and mean daily diet consumption was calculated as g food/100 g body weight/day. Food efficiency was not determined. Compound intake (mg/kg/day) values were calculated from the consumption and body weight data.

5. Water consumption:

Water consumption for each animal was determined weekly and was calculated as g water/100 g body weight/16-hour intervals (3:15 PM to 7:15 AM).

6. Ophthalmoscopic examination

Eyes were examined by slit lamp before the start of the study and at week 11.

7. Neurological tests

Neurological signs for spontaneous behavior, behavioral reaction to various manipulations, reflexes, and autonomous clinical signs were monitored before the start of the study and at week 11.

8. Blood was collected for hematology and clinical analysis from all survivors in all groups. Animals were not fasted prior to blood collection. Blood samples were taken at the end of treatment or at the end of the recovery period (29 days after removal of the test substance). The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*		
x	(Thromboplastin time)		
X	(Activated Partial		
X	Thromboplastin time)		
X	(Clotting time)		
X	(Prothrombin time)		
X			

* Required for subchronic studies based on Subdivision F Guidelines

Note: In addition to the above tests, methemoglobin was measured in the vehicle and high-dose groups and reticulocyte counts were only performed on vehicle and high-dose groups unless an effect was observed on these parameters.

b. Clinical Chemistry:

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)	X	Triglycerides
	Cholinesterase (ChE)	X	Serum protein electrophores
	Creatine phosphokinase	X	Total lipids
X	Lactic acid dehydrogenase (LDH)	X	Uric acid
X	Serum alanine amino-transferase (SGPT)*		
X	Serum aspartate amino-transferase (SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

8. Urinalysis*

Urine was collected over a 16-hour period from animals for whom water and feed had been removed. For the main study groups, urine was collected over days 82-83 (males) or days 78-79 (females). For the recovery groups, urine was collected over days 21-22 (males) or days 17-18 (females). The CHECKED (X) parameters were examined.

X	Color		
X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)	X	Nitrate
X	Protein	X	Urobilinogen
		X	Ascorbic acid

* Not required for subchronic studies
Note: Sediments were only examined for main and recovery group high-dose animals.

8. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*		Spinal cord (3 levels)[T]
X	Stomach*	X	Lymph nodes*		
X	Duodenum*	XX	Spleen*	XX	Pituitary*
X	Jejunum*	XX	Thymus*	X	Eyes (optic n.)[T]
X	Ileum*				
X	Cecum*		UROGENITAL		GLANDULAR
X	Colon*	XX	Kidneys*+		
X	Rectum*	X	Urinary bladder*	XX	Adrenal gland*
XX	Liver*+	XX	Testes*+		Lacrimal gland[T]
	Gall bladder*	X	Epididymides	X	Mammary gland[T]
X	Pancreas*	X	Prostate	X	Parathyroids*++
		XX	Seminal vesicle	XX	Thyroids*++
	RESPIRATORY	XX	Ovaries		
X	Trachea*	X	Uterus*		OTHER
XX	Lung*			X	Bone
	Nose			X	Skeletal muscle
	Pharynx			X	Skin
	Larynx			X	All gross lesions and masses*

* Required for subchronic studies based on Subdivision F Guidelines
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
T = required only when toxicity or target organ
In addition, the diaphragms were collected.

II. RESULTS

A. Observations :

1. Toxicity - No compound-related effects clinical findings were observed.

2. Mortality - No unscheduled deaths occurred.

B. Body weight and weight gain: Treatment with Hoe 061517 had no adverse effect on body weight.

C. Food consumption and compound intake:

1. Food consumption - Food consumption in the males and females of all dose groups was unaffected by treatment.

2. Test Article Intake - The mean intake values of Hoe 061517 calculated using nominal dietary levels for animals receiving test diets containing 0, 400, 1600 or 6400 ppm were reported as 0, 34, 127 or 546 mg/kg/day (males) or 0, 36, 141 or 570 mg/kg/day (females), respectively. Adjustments made by our reviewers for the actual concentrations of Hoe 061517 in the feed indicated compound intake values of 30, 102 or 420 mg/kg/day (males) and 32, 113, or 439 mg/kg/day (females).

3. Food efficiency - Food efficiency values were not determined.

D. Water consumption: Water consumption was unaffected by treatment with the test material. Although significant decreases in water consumption were recorded at various observation intervals in both the high-dose males and females, the results were generally sporadic and the mean relative intake over the entire 13-week treatment period showed no significant differences from control.

E. Ophthalmoscopic examinations - No treatment-related effects on the appearance of the eyes were observed at any examination period.

F. Blood work:

Hematology - No treatment-related effects on hematology parameters were observed. The significant increases in the reticulocyte counts for mid- and high-doses males of the main study groups and mid-dose males of the recovery group were considered by the study authors to be within the range of normal biological variation. Our reviewers also noted the lack of a dose response.

Clinical Chemistry - There was no indication of a compound-related effect on clinical chemistry parameters. Although significantly different from control results were recorded for several serum electrophoresis parameters in both the male and the female rats, the values were within historical ranges for male and female rats of this strain and age group.

G. Urinalysis - No treatment-related effects were observed.

H. Sacrifice and Pathology:

Organ weight - Significant changes in organ weights were sporadic. The study authors did note, however, that the absolute liver weights for high-dose males (main study and recovery groups) were increased (≈4 and 13%, respectively). The liver weight increase for the recovery group males was significant ($p<0.05$) Slight increases were also seen in the relative (to-body) male liver weights (Table 2). Additionally, very slight increases were observed in the absolute and relative high-dose female liver weights (main study group) but not in the recovery group. These marginal increase were not considered by the investigators to be related to exposure to Hoe 061517. Our reviewers noted, however, that marginal increases in the liver weights of females receiving 5000 ppm of the test material in the diet for 28 days were also recorded for the preliminary range-finding study. No comment was made by the study authors regarding the similar increases in both the absolute and relative kidney weights for high-dose males and females of both the main study and recovery groups (Table 3).

Gross pathology - No treatment-related necropsy findings were observed.

Microscopic pathology - The report stated that no treatment-related microscopic findings were observed. The presence of small disseminated Kupffer cell proliferates at terminal sacrifice in 5/10, 3/10 or 6/10 males administered 400, 1600 or 6400 mg/kg, respectively versus 2/10 in the control group was, however, noted by our reviewers. Similar observations were recorded in the high-dose recovery males (6/10 with small disseminated Kupffer cell proliferates versus 2/10 in control). Liver cell hypertrophy was also found in single high-dose females of the main study and recovery groups.

III. **DISCUSSION:**

Review of the final report and supporting data indicated that the conduct and design of the study were adequate and the reporting of the results was accurate. The marginally increased absolute and relative liver (to-body) weights in the high-dose males of the main study and recovery phase were not considered to be toxicologically relevant by the investigators because the finding did not correlate with other changes in the liver. As stated earlier by our reviewers, similar marginal increases in the liver weights of females receiving 5000 ppm of the test material in the diet for 28 days were recorded for the preliminary range-finding study. Additionally, our reviewers noted the presence of small Kupffer cell proliferates in the livers of 60% of the high-dose males of both the main and

recovery phase study groups versus a 20% incidence rate in the vehicle control rats. Similarly, a marginal (≈25%) increase in the reticulocyte counts for the main study high-dose males and for the recovery phase males (≈14%) receiving 6400 ppm was seen by our reviewers. Individually, none of these minimal changes are indicative of a compound effect; when viewed collectively, they do suggest an effect of treatment with Hoe 061517. This response is similar to that found in mice administered estrogen (Rouiller, 1964)[1]. In mice, estrogen stimulation of the phagocytic capacity of Kupffer cells in the liver is associated with cellular proliferation and increased liver weight. The marginal effects on kidney weights discussed above were not correlated with any pathological changes in the kidneys. Based on these considerations, we disagree with the study authors' assessment that the NOEL for Hoe 061517 was 6400 ppm. We conclude that the NOEL was 1600 ppm based on the marginal changes in male liver weights combined with the increased incidence of small Kupffer cell proliferates and increased reticulocyte counts in the male rats at 6400 ppm. Whether these findings are indicative of toxicologically significant adverse effects is not clear.

The LOEL and NOEL were, therefore, set at 6400 and 1600 ppm, respectively.

B. Study deficiencies: Although results from the analysis of test diets were outside of the acceptable range, actual dosages to the animals could be calculated. Hence, this deficiency probably did not alter the outcome of the study.

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[1]Rouiller, Ch. (1964). The Liver: Morphology, Biochemistry, Physiology. Vol. II. Academic Press, New York and London. p. 58.

Table 2. Liver Weights (Absolute and Relative) of Wistar Rats Ingesting Hoe 061517 in the Diet for 13 weeks[a,b]

	Dietary Level (ppm)			
Tissue	0	400	1600	6400
		MALES (PRIMARY GROUPS)		
Body weight (g)	404	395	396	401
Liver				
Absolute (g)	12.83	12.25	12.86	13.36 (4%)[c]
Relative (to Body Weight %)	3.18	3.10	3.24 (2%)	3.32 (4%)
		MALES (RECOVERY GROUPS)		
Body weight (g)	416	--	413	435
Liver				
Absolute	12.30	--	12.82 (4%)	13.85* (13%)
Relative (to Body Weight %)	2.96	--	3.09 (4%)	3.20 (8%)
		FEMALES (PRIMARY GROUPS)		
Body weight (g)	229	233	235	229
Liver				
Absolute	7.26	7.56 (4%)	7.60 (5%)	7.57 (4%)
Relative (to Body Weight %)	3.17	3.24 (2%)	3.24 (2%)	3.27 (3%)
		FEMALES (RECOVERY GROUPS)		
Body weight (g)	235	--	232	234
Liver				
Absolute	7.47	--	7.44	7.31
Relative (to Body Weight %	3.18	--	3.20	3.12

[a] Data extracted from Study # 87.0721 Organ Weight Summaries; pp. 121-132.
[b] N = 10 for all groups
[c] Values in () = percent increase over control.

* Significantly increased ($p \leq 0.05$) compared to control.

Table 3. Kidney Weights (Absolute and Relative) of Wistar Rats Ingesting Hoe 061517 in the Diet for 13 weeks[a,b]

Tissue	Dietary Level (ppm) 0	400	1600	6400
		MALES (PRIMARY GROUPS)		
Body weight (g)	404	395	396	401
Kidney				
Absolute (g)	2.22	2.15	2.21	2.39 (8%)[c]
Relative (to Body Weight %)	0.55	0.55	0.56	0.59* (7%)
		MALES (RECOVERY GROUPS)		
Body weight (g)	416	--	413	435
Kidney				
Absolute	2.11	--	2.29 (9%)	2.35 (11%)
Relative (to Body Weight %)	0.52	--	0.56 (8%)	0.54 (4%)
		FEMALES (PRIMARY GROUPS)		
Body weight (g)	229	233	235	229
Kidney				
Absolute	1.34	1.29	1.36	1.47 (10%)
Relative (to Body Weight %)	0.58	0.56	0.58	0.63 (9%)
		FEMALES (RECOVERY GROUPS)		
Body weight (g)	235	--	232	234
Kidney				
Absolute	1.34	--	1.34	1.46 (9%)
Relative (to Body Weight %	0.57	--	0.58	0.63 (11%)

[a] Data extracted from Study # 87.0721 Organ Weight Summaries; pp. 121-132.
[b] N = 10 for all groups
[c] Values in () = percent increase over control.

* Significantly increased ($p<0.05$) compared to control.

012180

[GLUFOSINATE-AMMONIUM METABOLITE] Subchronic Oral Study 82-1(a)

EPA Reviewer: Nancy McCarroll
Review Section III,
Toxicology Branch II (7509C)
EPA Secondary Reviewer:
Whang Phang, Ph.D.
Review Section III,
Toxicology Branch II (7509C)

Signature: Nancy E. McCarroll
Date: 3/4/97

Signature: Whang Phang
Date: 3/5/97

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity [Feeding]-[Mice]; OPPTS 870.3100 (rodent) [§82-1 (a)]

DP BARCODE: D229929 SUBMISSION CODE: S509558
P.C. CODE: 128850 TOX. CHEM. NO.: 580I (Metabolite)
MRID No.: 44076207

TEST MATERIAL (PURITY): Hoe 061517 (99.8%)

SYNONYMS: Code: Hoe 061517 0Q ZC99 0005; 3-methylphosphinico-propionic acid; metabolite of Hoe 039866 (Glufosinate-ammonium)

CITATION: Ebert, E. and Leist, K.H. (1989); Hoe 061517 - Substance Technical (Code: Hoe 061517 0Q ZC99 0005) Subchronic Oral Toxicity (13 Week Feeding Study) in the NMRI Mouse. Pharma Research Toxicology and Pathology Hoechst Aktiengesellschaft, Frankfurt am Main, Germany. Study No. 88.0693: Report No. A41762; Study Completion Date: June 16, 1989; MRID No.:44076207. Unpublished.

SPONSOR: AgrEvo USA Co., Wilmington, DE

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID No.44076207), Hoe 061517 (99.8%) was administered in the diet for 13 weeks to male and female NMRI mice at dose levels of 0, 320, 1600, 3200 or 8000 ppm (0, 46, 209, 496 or 1121 mg/kg/day in the males and 0, 47, 220, 561 or 1340 mg/kg/day in the females, respectively. All groups consisted of 10 mice/sex/dose. Glutamine synthetase activity was not measured.

Treatment with Hoe 061517 had no adverse effects on survival, body weight, hematology or urinalysis. However, a marginal but significant increase in the relative high-dose female kidney weights and dose-related significant decreases in serum uric acid levels were observed in the mid- and high-dose males (16 and 40% of control, respectively). In the females, nondose-related ≈33 or 25% decreases in uric acid levels were seen at 3200 or 8000 ppm, respectively. There was, however, no evidence of gross or microscopic lesions associated with exposure to Hoe 061517. Whether the marginal kidney weight changes or decreased uric acid

levels are indicative of a toxicologically significant adverse effect is not clear. **In disagreement with the study authors, however, the LOEL was set at 8000 ppm based on the decreased serum uric acid levels. The NOEL was established at 3200 ppm.**

This subchronic toxicity study is classified **acceptable (Nonguideline)** as it is not a required study. It is, however, acceptable for the purposes for which it was intended as a special study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Hoe 061517 technical
   Description: White crystalline solid
   Lot/Batch #: Sample No. Roe 15408
   Purity: 99.8%
   Stability of compound: Reported to be stable in the diet for at least 30 days.
   CAS #: Not provided.
   Structure: Not provided.
   Other provided information: None

2. Vehicle and/or positive control: Basal diet (Altromin 1321 rat and mouse diet)

3. Test animals: Species: NMRI mice Strain: Hoe:NMRKf(SPF71)
   Age at study initiation : ≈4 weeks
   Weight (study initiation): 17.7 ± 0.7 g (♂); 17.0 ±0.7 g (♀)
   Source: Hoechst AG, Kastengrund, Ger.
   Housing: 5/cage
   Diet: Altromin 1321 rat and mouse diet was available ad libitum except during urine sample collection.
   Water: Tap water was available ad libitum, except during urine sample collection.

   Environmental conditions:
   Temperature: 20-22°C
   Humidity: 50-70%
   Air changes: 6 times/hour
   Photoperiod: 12 hours/day
   Acclimation period: ≈7 days

B. STUDY DESIGN:

1. In life dates - start: 7/26-29/88 end: 10/25-28/88

2. Animal assignment

   Animals were assigned randomly to the test groups shown in Table 1.

TABLE 1: STUDY DESIGN

Test Group	Conc. in Diet (ppm)	Dose to Males (mg/kg/day)	Dose to Females (mg/kg/day)	No. of ♂	No. of ♀
Control	0	0	0	10	10
Low	320	46[a]	47[a]	10	10
Mid #1	1600	209	220	10	10
Mid #2	3200	496	561	10	10
High	8000	1121	1340	10	10

[a] Actual dosages to the animals based on daily intake and analytical determinations (calculated by our reviewers).

3. Diet preparation and analysis:

Premix batches of the test material in basal feed were prepared monthly and stored presumably at room temperature. The test compound in the premixes was reported to be 10x the final concentration. Final mixtures were prepared monthly by mixing the appropriate amount of premix with the basal feed. Each final test diet was analyzed for actual concentration. Stability at days 0, 7, 14 and 30 and homogeneity were measured once on the initial batch of the test diets.

Results -

Homogeneity/Stability Analysis:
Day 0: 71-98% Day 21: 73-92%
Day 7: 73-91% Day 30: 75-88%
Day 14: 74-101%

Concentration Analysis:

	Range	Average Values
Low-dose:	78-88% of nominal	83%
Mid-dose (#1):	70-90% of nominal	79%
Mid-dose (#2):	84-101% of nominal	95%
High-dose:	79-91% of nominal	87%

The analytical data indicated that the mixing procedures were adequate to determine the actual concentration of the test substance. However, the variance between nominal and average actual dosage to the animals (79-95%) was unacceptable. Therefore, the average values calculated by our reviewers were used to determine actual dosages to the animals.

4. Statistics: Body weight (320-and 1600-ppm treatment groups only) and organ weight data (relative and absolute) were evaluated using the parametric method of Dunnett and/or the distribution free method of Nemenyi/Dunnett. Hematology and clinical chemistry data were evaluated using the parametric method of Dunnett, the parametric method of Sidak, the distribution free method of Nemenyi/Dunnett and/or the distribution-free method of Nemenyi/Sidak. Urinalysis data (pH only) were analyzed using the distribution free method of Nemenyi/Dunnett and/or the distribution-free method of Nemenyi/Sidak. In all cases, significance was established at $p<0.05$.

C. METHODS:

1. Observations:

Animals were inspected for mortality and behavior twice daily. Other signs of toxicity including neurological disturbances, opacity of the refracting media of the eyes, damage to the oral mucosa, and impairment of dental growth were monitored weekly.

2. Body weight:

Animals were weighed once during the acclimation period and weekly, thereafter, throughout the course of study.

3. Food consumption and compound intake:

Food consumption for each animal was determined weekly and mean daily diet consumption was calculated as g food/100 g body weight/day. Food efficiency was not determined. Compound intake (mg/kg/day) values were calculated from the consumption and body weight data.

4. Water consumption:

Water consumption for each animal was determined weekly and was calculated as g water/100 g body weight/16-hour intervals (3:15 PM to 7:15 AM).

5. Ophthalmoscopic examination

Eyes were not examined.

8. Blood was collected for hematology and clinical analysis from all survivors in all groups at the termination of treatment. Animals were not fasted prior to blood collection. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*	X	Methemoglobin
	(Thromboplastin time)		
	(Activated Partial Thromboplastin time)		
X	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

Note: Methemoglobin determinations and reticulocyte counts were not performed on the low- or two intermediate-dose groups since no abnormalities were seen in the high-dose group.

b. Clinical Chemistry:

	ELECTROLYTES		OTHER
X	Calcium*++	X	Albumin*+
X	Chloride*+++	X	Blood creatinine*+++
	Magnesium	X	Blood urea nitrogen*+++
X	Phosphorus*++	X	Total Cholesterol+
X	Potassium*+++	X	Globulins+
X	Sodium*+++	X	Glucose*+
		X	Total bilirubin++
	ENZYMES	X	Total serum protein (TP)*+
X	Alkaline phosphatase (ALK)+	X	Triglycerides+
	Cholinesterase (ChE)	X	Serum protein electrophores+
	Creatine phosphokinase	X	Total lipids++
	Lactic acid dehydrogenase (LDH)	X	Uric acid++
X	Serum alanine amino-transferase (SGPT)*+		
X	Serum aspartate amino-transferase (SGOT)*+		
X	Gamma glutamyl transferase (GGT)+		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines.
+ Parameter was examined in all animals.
++ Parameter was examined in 5 males/group or 3-5 females/group.
+++ Parameter was examined in 5 males/group but was not examined in the females.

8. Urinalysis*

Urine was collected over a 16-hour period from animals for whom water and feed had been removed. Urine was collected over days 84-85 (males) or days 83-84 (females). The CHECKED (X) parameters were examined.

X	Color		
X	Appearance	X	Glucose
	Volume	X	Ketones
	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)	X	Nitrate
X	Protein	X	Urobilinogen
		X	Ascorbic acid

* Not required for subchronic studies.
Note: Sediments were not examined for the low- or two intermediate-group animals since no abnormalities were seen in the high-dose mice.

8. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue		**Aorta***	XX	Brain*
	Salivary glands*	XX	Heart*		**Periph. nerve***
	Esophagus*	X	Bone marrow*		Spinal cord (3 levels)[T]
X	Stomach*		**Lymph nodes***		
	Duodenum*	XX	Spleen*	X	Pituitary*
X	Jejunum*	X	Thymus*	X	Eyes (optic n.)[T]
	Ileum*				
	Cecum*		UROGENITAL		GLANDULAR
X	Colon*	XX	Kidneys*+		
	Rectum*	X	Urinary bladder*	XX	Adrenal gland*
XX	Liver*+	XX	Testes*+		Lacrimal gland[T]
	Gall bladder*	X	Epididymides		Mammary gland[T]
X	Pancreas*	X	Prostate		**Parathyroids***++
		XX	Seminal vesicle	X	Thyroids*++
	RESPIRATORY	XX	Ovaries		
X	Trachea*	X	Uterus*		OTHER
XX	Lung*				Bone
	Nose				Skeletal muscle
	Pharynx				Skin
	Larynx			X	All gross lesions and masses*

* Required for subchronic studies based on Subdivision F Guidelines
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

Note: Tissues from the organs **in bold**, which are required by guideline, were not taken.

II. **RESULTS**

A. Observations :

1. Toxicity - No compound-related effects clinical findings were observed.

2. Mortality - No unscheduled deaths occurred.

B. Body weight and weight gain: Treatment with Hoe 061517 had no adverse effects on body weight.

C. Food consumption and compound intake:

1. Food consumption - Food consumption in the males and females of all dose groups was unaffected by treatment.

2. Test Article Intake - The mean intake values of Hoe 061517 calculated using nominal dietary levels for male mice receiving test diets of 0, 320, 1600, 3200 or 8000 ppm were 0, 55, 264, 522 or 1288 mg/kg/day. The mean intake values for females receiving these same dietary concentrations were 0, 57, 279, 590 or 1540 mg/kg/day, respectively. Adjustments made by our reviewers for the actual concentrations of Hoe 061517 in the feed indicated compound intake values of 0, 46, 209, 496 or 1121 mg/kg/day (males) and 0, 47, 220, 561 or 1340 mg/kg/day (females).

3. Food efficiency - Food efficiency values were not determined.

D. Water consumption: Water consumption was unaffected by treatment with the test material.

E. Blood work:

Hematology - No treatment-related significant effects on hematology parameters were observed.

Clinical Chemistry - More than one-half of the parameters that were listed by the study authors were not examined in 10 animals/sex/group, and certain parameters (i.e., Na, K, Cl, creatine, and urea) were not evaluated in the females. Serum uric acid levels were decreased in the males to 16 and 40% of

control at 3200 and 8000 ppm (control: 231 μmoles/L, 3200 ppm: 194 μmoles/L, 8000 ppm: 137 μmoles/L); however, only the high dose-group value was significant. Nonsignificant and non-dose-related decreases in serum uric acid were seen in the females at 3200 ppm (33%↓) and at 8000 ppm (25%↓). Other significant findings in the males included a significant (≈27%) decrease in ASAT (high-dose group) and significant (≈43%) increases in ALK (high intermediate- and high-dose groups). In the females, significant but not dose-related increases in cholesterol levels were reported in all treatment groups. Other significant effects (i.e., ↓ triglycerides, ↓ total lipids and ↓ protein --high intermediate and/or high dose) were either marginally different from control or not dose-related. With the exception of the significant decrease in serum uric acid in the high-dose males, all other significant findings were reported to be within the range of biological variation. No historical control data were provided to support this claim.

G. Urinalysis - No treatment-related effects were observed.

H. Sacrifice and Pathology:

Organ weight - Relative (to-body) kidney weights were significantly increased in high-dose males and females (≈11 or 12% of control for males or females, respectively). The study authors discounted the significant relative kidney weight increase in the males and stated that the increase should be viewed in connection with the "somewhat lower body weight". Our reviewers agree with this assessment since these organ weight changes were well within the standard deviations. There were no other indications of a treatment-related response on organ weight data.

Gross pathology - No treatment-related necropsy findings were observed.

Microscopic pathology - Only incidental findings, which were not related to treatment with Hoe 061517, were observed.

III. DISCUSSION:

Review of the final report and supporting data indicated that the conduct and design of the study were adequate and the reporting of the results was accurate. The study authors considered the marginal increase in the relative kidney weight of high-dose females and the decreased serum uric acid levels in high-doses males to be effects of treatment with Hoe 061517 but of no toxicological significance in the absence of further toxicologic correlates. Nevertheless, our reviewers noted that

serum uric acid levels were decreased in the females at 3200 ppm (33%↓) and at 8000 ppm (25%↓). There was, however, no evidence of gross or microscopic lesions in the kidneys. Based on these considerations, we disagree with the study authors' assessment that the NOEL for Hoe 061517 was 8000 ppm. We conclude that the NOEL was 3200 ppm based on the decreased serum uric acid levels. Whether these findings are indicative of toxicologically significant adverse effects is not clear.

The LOEL and NOEL were, therefore, set at 8000 and 3200 ppm, respectively.

B. Study deficiencies: The following study deficiencies were noted:

No statistical analysis of the two highest dose group body weights was performed.

Na, K, Cl, Ca, P, creatinine, urea, total bilirubin, or total lipids were either not determined in the females or were not determined in all of the females.

Salivary glands, esophagus, doudenum, ileum, cecum, rectum, aorta, lymph nodes, peripheral nerves or parathyroids were not examined histologically.

It is not likely that the above deficiencies altered the outcome of the study or the interpretation of the results since none of these parameters were affected in other subchronic feeding studies conducted with the test article.

[GLUFOSINATE-AMMONIUM METABOLITE] Subchronic Oral Study 82-1(b)

EPA Reviewer: Nancy McCarroll
Review Section III,
Toxicology Branch II (7509C)
EPA Secondary Reviewer:
Whang Phang, Ph.D.
Review Section III,
Toxicology Branch II (7509C)

Signature: Nancy E. McCarroll
Date: 1/15/97

Signature: W Phang
Date: 1/16/97

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity [Feeding] - [Beagle Dogs]; OPPTS 870.3151 (nonrodent) [§82-1 (b)]

DP BARCODE: D229929 SUBMISSION CODE: S509558
P.C. CODE: 128850 TOX. CHEM. NO.: 580I (Metabolite)
MRID No.: 44076208

TEST MATERIAL (PURITY): Hoe 061517 (99.6%)

SYNONYMS: Code: Hoe 061517 0Q ZC99 0003; 3-methylphosphinico-propionic acid; metabolite of Hoe 039866 (glufosinate-ammonium)

CITATION: Brunk, R. (1988); Hoe 061517 - Substance Technical (Code: Hoe 061517 0Q ZC99 0003) Testing for Toxicity by Repeated Oral Administration to Beagle Dogs (3-Month Feeding Study). Pharma Research Toxicology and Pathology Hoechst Aktiengesellschaft, Frankfurt am Main, Germany. Study No. 87.0722: Report No. A39880; Study Completion Date: June 21, 1988; MRID No.:44076208. Unpublished.

SPONSOR: AgrEvo USA Co., Wilmington, DE

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID No.44076208), Hoe 061517 (99.6%) was administered in the diet for ≈15 weeks to male and female beagle dogs at dose levels of 100, 400 or 1600 mg/kg (equivalent daily intakes could not be determined because of the inadequacies in the analytical data). Groups consisted of six dogs/sex/dose in the vehicle control and mid- and high-treatment groups and four dogs/sex in the low-treatment group. Two dogs/sex/dose from the vehicle control and mid- and high-treatment groups were retained following treatment for a 4-week recovery period. In addition to standard testing, samples of livers, kidneys and brains of all animals were analyzed for glutamine synthetase activity. Hearing tests, neurological examinations hepatic function tests (i.e., bromsulphthalein retention) and renal function test (i.e., phenolsulfonphthalein elimination) were also conducted.

Treatment with Hoe 061517 had no adverse effects on survival, body weight, hematology, clinical chemistry, urinalysis, liver or renal function, glutamine synthetase activity, or cause hearing disfunction or neurological changes. Similarly, there was no evidence of gross or microscopic lesions associated with exposure to Hoe 061517. **However, neither a LOEL nor a NOEL could be established because of numerous study deficiencies (see Section III, Discussion for details).**

This subchronic toxicity study is classified **Unacceptable (Nonguideline)** as it is not a required study. It is, however, unacceptable for the purposes for which it was intended as a special study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Hoe 061517 technical
   Description: Not listed
   Lot/Batch #: Sample No. Roe 15109
   Purity: 99.6%
   Stability of compound: Stability of the test substance at room temperature in corn meal was reported to be guaranteed for 4 months.
   CAS #: Not provided.
   Structure: Not provided.
   Other provided information: None

2. Vehicle and/or positive control: Corn meal (premix base)

3. Test animals: Species: Pure bred beagle dogs of the Hoe:BEAK strain (Hoechst breed)
   Mean age at study initiation: 7 months
   Weight at initiation: 11.2-14.3 kg (♂); 10.5-14.0 kg (♀)
   Source: In-house
   Housing: Individual kennels
   Diet: Vipromix feed was available daily (feeding took place at 1:00 PM with daily portions of 1000 g given to the males and 800 g given to the females).
   Water: Tap water was available ad libitum

   Environmental conditions:
   Temperature: 18°C
   Humidity: Not reported
   Air changes: Not reported
   Photoperiod: Not reported
   Acclimation period: Not applicable

B. STUDY DESIGN:

1. In life dates - start: 11/7/87 end: 3/24/88

2. Animal assignment

   Animals were assigned (method not reported) to the test groups in Table 1.

TABLE 1: STUDY DESIGN

Test Group	conc. in Diet (ppm)	Dose to Animal (mg/kg/day)	Male[a]	Female[a]
Control	0	0	6	6
Low	100	[b]	4	4
Mid	400	[b]	6	6
High	1600	[b]	6	6

[a] At the end of treatment, two males and two females in the vehicle and mid-and high-dose groups were retained for a 4-week recovery period.

[b] The actual concentrations fed to the animals could not be determined from the available data (see Diet Preparation and analysis, section B.3 of this DER for details).

3. Diet preparation and analysis:

Premix batches of the test material in corn meal were prepared monthly and stored at room temperature. Final mixtures were prepared daily by stirring the appropriate amount of premix into the dog feed. Each premix batch was analyzed for homogeneity and actual concentration in corn meal. Stability and content of the test diets was measured once and only after the completion of the study on a single batch of the final dietary mixture (premix + basal diet) containing a target concentration of 400 mg/kg).

Results -

Stability and Content Analysis (400 mg/kg final test diet): Ranged from an average high of 82% of target immediately after preparation to an average low of 78% 24 hours post-preparation.

Concentration/Homogeneity Analysis (premixes): Ranges of percent nominal were as follows:

		Average %
· low-dose premix:	102-107%	105%
· mid-dose premix:	75-93%	81%
· high-dose premix:	72-94%	85%

The analytical data indicated that the mixing procedures were not adequate to determine the actual concentration of the test substance in the test diets for the following reasons:

Only premix samples were analyzed for content during the course of the study. Based on the values obtained from the analysis of the premixes, we are unable to determine with any confidence whether the actual doses fed to the dogs contained 100% of the achieved concentrations in the premixes or whether only a portion of the achieved concentrations were available to the animals.

Additionally, the performance of the stability and content analysis on an actual test diet after completion of the study, and only on a single mid-dose batch provided no confidence in the accuracy of diet preparation.

4. Statistics: Body weight (final only) and organ weight data were combined for both sexes and evaluated using the parametric method of Dunnett, the distribution free method of Nemenyi/Dunnett and/or by a two-sided T-test. Hematology data were combined for both sexes and evaluated using the parametric method of Sidak, the distribution-free method of Nemenyi/Sidak, the parametric method of Dunnett and/or the distribution free method of Nemenyi/Dunnett. Clinical chemistry data were evaluated using the parametric method of Sidak, the distribution-free method of Nemenyi/Sidak, 2-sided t-test and/or 2-sided Wilcoxon test. Calcium, total bilirubin, urea, SGOT, SGPT, alkaline phosphatase, and serum electrophoresis values were analyzed by sex; all other clinical chemistry values were combined for both sexes prior to statistical analysis. Urinalysis data (specific gravity and pH only) were evaluated combined for both sexes using the distribution-free method of Nemenyi/Sidak. In all cases, significance was established at $p<0.05$.

Note: Statistical analysis was not performed on the glutamine synthetase data.

C. METHODS:

1. Observations:

Animals were inspected for mortality and behavior twice daily. Other signs of toxicity were monitored once daily.

2. Body weight:

Animals were weighed weekly.

3. Food consumption and compound intake:

Food consumption for each animal was recorded daily and individual diet consumption data were assumed to be equivalent to ppm based on the 100% daily consumption reported for all groups of both sexes.

4. Ophthalmoscopic examination

Eyes were examined before the start of the study, after ≈6 weeks, before termination of the study and "towards" the end of the recovery period.

5. Neurological tests

Neurological signs for excitability (pupillary, blink, flexor, patellar, anal and cutaneous reflexes) and postural reactions (extensor, thrust, visual and tactile placing and righting) were monitored before the start of the study, after ≈6 weeks, before termination of the study and "towards" the end of the recovery period.

6. Hearing tests

Hearing tests were conducted before the start of the study, after ≈6 weeks, before termination of the study and "towards" the end of the recovery period.

7. Dental examinations

Dental examinations were performed before the start of the study, after ≈6 weeks, before termination of the study and "towards" the end of the recovery period.

8. Blood was collected for hematology and clinical analysis from all survivors in all groups. Animals were fasted for ≈18-20 hours prior to blood collection. Blood samples were taken before the start of the study, after ≈6 weeks, before termination of the study and "towards" the end of the recovery period. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc. (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*		
x	(Thromboplastin time) (Activated Partial Thromboplastin time) (Clotting time)		
X	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

Note: In addition to the above tests, methemoglobin was measured at study completion in all animals of the main study groups but not in the recovery groups.

b. Clinical Chemistry:

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
X	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)	X	Triglycerides
	Cholinesterase (ChE)	X	Serum protein electrophores
	Creatine phosphokinase	X	Iron
X	Lactic acid dehydrogenase (LDH)	X	Uric acid
X	Serum alanine amino-transferase (SGPT)*		
X	Serum aspartate amino-transferase (SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

8. Urinalysis*

Urine was collected from animals fasted for an unspecified time before the start of the study, after ≈6 weeks, before termination of the study and "towards" the end of the recovery period. The CHECKED (X) parameters were examined.

X	Color		
X	Appearance	X	Glucose
	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen

* Not required for subchronic studies

8. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)[T]
X	Stomach*	X	Lymph nodes*		
X	Duodenum*	XX	Spleen*	XX	Pituitary*
X	Jejunum*	XX	Thymus*	X	Eyes (optic n.)[T]
X	Ileum*				
X	Cecum*		UROGENITAL		
X	Colon*	XX	Kidneys*+		GLANDULAR
X	Rectum*	X	Urinary bladder*	XX	Adrenal gland*
XX	Liver*+	XX	Testes*+		Lacrimal gland[T]
X	Gall bladder*	XX	Epididymides	X	Mammary gland[T]
XX	Pancreas*	XX	Prostate	XX	Parathyroids*++
			Seminal vesicle	XX	Thyroids*++
	RESPIRATORY	XX	Ovaries		
X	Trachea*	XX	Uterus*		OTHER
XX	Lung*			X	Bone
	Nose			X	Skeletal muscle
	Pharynx			X	Skin
	Larynx			X	All gross lesions and masses*

* Required for subchronic studies based on Subdivision F Guidelines
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
T = required only when toxicity or target organ
In addition, tonsils and sections of the diaphragmatic muscle were collected.

9. Additional Testing:

Hepatic Function Test Bromsulphthalein (BSP) was measured at 2 minutes and at 45 minutes after BSP injection in all dogs before the start of the study, after ≈6 weeks, at termination of the study and before the end of the recovery period. Note: Data were presented for only one of the two sampling times and the time was not specified.

Renal Function Test Phenolsulfonphthalein (PSP) was measured at 10 minutes and at 30 minutes after PSP injection in all dogs before the start of the study, after ≈6 weeks, at termination of the study and before the end of the recovery period.

Glutamine Synthetase Activity: Samples of the liver, kidneys and brain for all animals were analyzed for glutamine synthetase (GS). Samples were stored frozen until analysis. GS activity was determined according to a "modified version" of the method of Elliott (1955). The analytical method was photometric and was based on the formation of Fe^{3+}-hydroxamate complex catalyzed by GS. GS activity in each sample was determined using a gamma-glutamylhydroxamate calibration curve. Data were expressed as nkat/mg protein; we assume that the unit would be equivalent to nmoles/mg protein; however, since nkatals are generally expressed relative to time, we are not sure that the conversion is correct.

II. RESULTS

A. Observations :

1. Toxicity - The summary of clinical findings indicated no compound-related effects. Individual data were, however, not provided.

2. Mortality - No unscheduled deaths occurred.

B. Body weight and weight gain: Treatment with Hoe 061517 had no adverse effect on body weight.

C. Food consumption and compound intake:

1. Food consumption - Food consumption in the males and females of all dose groups was unaffected by treatment. Our reviewers noted, however, the unusual consistency of the individual daily food consumption data (see Study Report pp.220-267). Without exception, all male rats in all treatment groups consumed 1000 g of feed and all female rats in each experimental group consumed 800 g of feed every day for the entire ≈15 weeks of feeding test diets. The same trend of 100% feed consumption continued throughout the 4-week recovery period. Our reviewers noted, however, that despite the reported 800 mg/day consistent food consumption, a female control (#4273) lost 100 gm of body weight during the study.

2. Test Article Intake: The mean intake values of Hoe 061517 could not be calculated using nominal dietary levels because of the uncertainties regarding the actual concentrations of the test substance in the experimental diets (see Section B.3., Diet Preparation and Analysis for details).

3. Food efficiency - Food efficiency values were not determined.

D. Ophthalmoscopic examinations - No treatment-related effects on the appearance of the eyes were observed at any examination period.

E. Hearing/Dental examinations - No treatment-related effects were observed.

F. Blood work:

Hematology - No treatment-related significant effects on hematology parameters were observed.

Clinical Chemistry - There was no indication of a compound-related effect on clinical chemistry parameters. Although significantly different from control results were recorded for several parameters in both the male and the female dogs, the values did not showed a consistent pattern of time or dose dependency.

G. Urinalysis - No treatment-related effects were observed.

H. Additional Studies:

Hepatic and Renal Function - Although the protocol stated that BSP levels would be determined 2 and 45 minutes post-injection, data were only presented from a single time period; the interval for the presented data was also not reported. BSP levels in the males remained relatively constant in all treatment groups compared to control throughout the study. The significant changes in the BSP levels for the high-dose group (combined for both sexes) that occurred in samples taken at termination of the main study and the recovery phase of testing were associated with the erratic values obtained with the high-dose females. The findings were not considered to be of toxicological significance since BSP levels declined at 6 weeks, increased at main study termination and decreased after recovery. Similarly, PSP data showed no toxicologically relevant delay in the elimination of PSP in any treatment group at any sampling time.

Glutamine Synthetase (GS) Activity: Summarized data from the determination of GS activity in the livers, kidneys and brains of treated males and females are presented in Table 2. As shown, no GS inhibition was seen in the liver or kidneys of the male dogs. Values for brain GS activity in the males were erratic with ≈49% inhibition in the low-dose group, ≈15% inhibition in the mid-dose group and ≈25% inhibition in the high-dose group. Erratic data were also obtained for female

liver enzyme levels (i.e, 29, 26 or 35% GS inhibition in the 100-, 400- or 1600-dose groups, respectively). No clear effects on either kidney or brain GS activity were observed in the treated dogs of both sexes. Based on the data, the author of the GS report stated that the test material did not cause inhibition of GS activity in the selected organs. The author of this report also stated the following: "Whereas the glutamine synthetase activity in the liver and brain of the test animals was easily measurable, it was difficult to detect enzyme activity in the kidneys. The measured values were situated around the detection limit and thus offer no basis for a definitive statement."

H. Sacrifice and Pathology:

Organ weight - No treatment-related effects on either the absolute or relative (to body weight) organ weights for the male dogs was seen. Neither the ≈50% absolute and relative prostate weight decrements relative to control noted by our reviewers for the high-dose males were significant. In the females, the significantly reduced absolute uterine weight for the high-dose group was stated by the investigators to be caused by estrus presumably in the control group females. Other significant organ weight differences were sporadic and not dose-related. We note, however, that organ weight data were combined by sex for the various treatment groups.

Gross pathology - No treatment-related necropsy findings were observed.

Microscopic pathology - No treatment-related microscopic findings were observed.

III. **DISCUSSION:**

The numerous study deficiencies listed below preclude acceptance of the findings as definitive evidence that exposure of dogs to dietary levels of Hoe 061517 for ≈15 weeks is without toxicological significance:

The submitted analytical data on content of Hoe 061517 in the experimental diets were not adequate to determine the actual concentration of the test substance available to the animals.

Individual clinical observation data were not provided.

The unusual consistency of the individual daily food consumption data (i.e., 100% feed consumption for both sexes every day for the entire ≈15 weeks of feeding test diets as well as during the recovery period) is an unexpected finding and raises concerns regarding the accuracy of feed consumption measurements. In

addition, the average daily feed consumption for dogs generally ranges from 240-500 gm/day. Hence, the daily consumption of 1 kg of feed for males and 800 gm of feed for females appears to be unusually high.

The statistical analysis of numerous study parameters on data combined for both sexes in not scientifically valid.

The unit used to express GS values (nkat/mg protein) appears to be incomplete with respective to time. However, data presented for this endpoint in similar studies conducted with the parent compound (see MRID No. 00142444) expressed GS activity as µmol product/mg protein/20 minutes. We assume, therefore, that the values for GS activity used in the currently reviewed study would be equivalent to nmol/mg protein/20 minutes since the reaction time was 20 minutes. If our assumption is correct, the levels of liver GS that were detected were exceeding low (e.g., 1.4-1.5 µmol/mg protein/20 min. in control males and females -- MRID No. 0014244 versus 0.2-0.3 nkat/mg protein in the control males and females of this study--see Table 2). We conclude, therefore, that the low sensitivity of the methodology cast doubts on the validity of the findings and precludes an independent assessment of the potential of Hoe 061517, if any, to cause an inhibitory effect on this enzyme.

Based on the above considerations, neither a LOEL nor a NOEL can be established for this study.

B. Study deficiencies: See above.

Table 2. Glutamine Synthetase Activity (nkat/mg protein) in Dogs Ingesting Hoe 061517 in the Diet for ≈15 weeks[a,b]

Tissue	Dietary Level (ppm) 0	100	400	1600
		MALES		
Liver				
Week 15	0.23	0.24	0.37	0.38
Recovery Group[c]	0.29	--	0.20	0.33
Kidney				
Week 15	0.02	0.02	0.02	0.02
Recovery Group	0.03	--	0.03	0.03
Brain				
Week 15	1.50	0.77	1.27	1.12
Recovery Group	1.24	--	1.41	1.04
		FEMALES		
Liver				
Week 15	0.34	0.24	0.25	0.22
Recovery Group[c]	0.27	--	0.23	0.30
Kidney				
Week 15	0.02	0.02	0.01	0.02
Recovery Group	0.02	--	0.01	0.01
Brain				
Week 15	1.28	1.28	1.09	1.23
Recovery Group	1.11	--	1.10	1.16

[a] Data extracted from Study # 87.0722, Glutamine synthetase testing; pp. 292-294.
[b] N = 4 for all groups except the recovery groups for which N = 2.
[c] Recovery group samples were taken ≈4 weeks after the termination of treatment.

Note: The data were not evaluated statistically.

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Chemical:	**Glufosinate-ammonium**
PC Code:	**128850**
HED File Code	***13000 Tox Reviews***
Memo Date:	**03/07/97**
File ID:	**TX012180**
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